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FREEZING TEMPERATURES OF HIGH-PURITY IRON AND OF SOME STEELS

By Wm. F. Roeser and H. T. Wensel

ABSTRACT

The freezing temperature of high-purity iron (99.99+ percent) in an atmosphere of helium was measured with an optical pyrometer and found to be 1,539° ±1° C. The same samples of iron were found to freeze 1° C lower in an atmosphere of hydrogen. In addition, the initial freezing temperatures in an atmosphere of helium of some irons of lesser purity and of some steels were measured to determine the effects of various impurities and alloying elements. The results of measurements on 23 samples of irons and steels, together with the chemical analyses, are reported. The freezing-point depressions of the various elements are listed in a table which may be used to calculate the initial freezing temperatures (liquidus points) of iron containing many combinations of 17 elements, which include all those generally found in commercial irons and steels.

The samples containing less than 0.1 percent of impurities were melted in beryllium oxide crucibles and the other samples, in all but two cases, were melted in crucibles of aluminum oxide. Temperatures were determined by means of an optical pyrometer sighted into refractory blackbodies immersed in the freezing metal.

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I. INTRODUCTION

The wide variety of purposes for which iron and steel are used makes their properties of the greatest importance. Furthermore numerous alloys with special properties are required to satisfy the needs of modern industry. It is for this reason that the effect of various impurities and alloying elements upon the properties of iron is of such great interest. The effect of such constituents upon any property of pure iron can be established only after the value of that particular property has been determined for pure iron. One property,

which is of particular interest to those investigating or producing iron or steel, is the melting, or freezing, point.

Although many values for the melting point of iron have been reported, there is still some uncertainty regarding the melting point of pure iron, because iron of high purity has not been available for the measurements and because no determination under entirely satisfactory experimental conditions has been reported. Even after a relatively pure iron has been produced and its purity established, precautions must be taken to maintain its purity during the measurement of the melting point, because it is so readily contaminated by many crucible materials and gases during melting [1].[1]

II. PREVIOUS WORK

A summary of the various determinations of the melting or freezing point of iron since 1904 is given in table 1. Many of the earlier determinations are included primarily for their historical interest. In comparing the reported values in table 1 with one another, it should be borne in mind that in nearly all of these investigations one or more of the following conditions prevailed: (1) The investigators were primarily interested in establishing phase equilibrium or constitutional diagrams and were not primarily interested in an accurate determination of the melting point of iron. (2) The impurities reported were so great and so diverse that the various results can scarcely be considered as determinations of the melting point of the same material. (3) No consideration was given to gases, which have a significant effect upon the melting point. (4) The iron was subjected to contamination, particularly by gases, during the determination. (5) The temperature-measuring instruments used in the various investigations were not calibrated on the same basis.

If a complete chemical analysis, including gases, had been given in each case, the value which each observer would have obtained on his particular temperature scale with metal of any specified purity could be computed from the effects of various impurities upon the melting point, but the analyses in most cases are inadequate to give any significance to such a calculation. It was decided to accept the values reported as applying to the melting point of the particular iron used in the particular atmosphere employed, and to reduce the reported values of temperature to a common basis as far as possible. However, even this adjustment has certain limitations.

In most of the determinations listed in table 1, the reported value is based upon a calibration of the temperature-measuring instrument at either the melting point of nickel or the melting point of palladium with various values for each. The adjustment of the reported values to the same temperature scale requires the selection of one particular value for the melting point of nickel and another for the melting point of palladium. The two values selected for these two melting points must be on the same temperature scale. The procedure is questionable because there is no reason to believe that all the nickel or all the palladium used in these calibrations melted at the same temperature and because there is no way of determining what the respective values were.

[1] Figures in brackets indicate the literature references at the end of this paper.

TABLE 1.—*Determinations of the melting or freezing temperatures of iron*

Observers	Reference	Year	Reported impurities	Crucible material	Atmosphere	Value reported	Type of instrument used	Basic calibration point	Reported values adjusted for Ni-1,455° C Pd-1,554 Pt-1,773
			% by wt			°C		°C	°C
Carpenter and Keeling	[2]	1904	0.11	Magnesia	CO	1,505	Thermocouple	Extrapolated above 1,000° C	
Guertler and Tammann	[3]	1905	.2 to .6	Porcelain	N_2	1,555 ±5	do	Ni-1,484	1,521
Isaac and Tammann	[4]	1907	.29		N_2	1,527 ±5	do	Ni-1,451	1,532
G. K. Burgess	[5]	1907	.05		H_2	1,505	Optical	Pt-1,753	1,517
H. Karkort	[6]	1907	.73	Magnesia	CO	1,504	Thermocouple	Pd-1,535	1,532
R. Sahmen	[7]	1906	.27	Porcelain	N_2	1,532	do	Ni-1,451	1,537
B. Saklatwalla	[8]	1908		Magnesia	N_2	1,510	do	Cu-no value	
A. Müller	[9]	1909		Magnesite	Vacuo	1,485 to 1,525	do		
N. Konstantinow	[10]	1910	.20	do	Under $BaCl_2$	1,514	do	Ni-1,451	1,519
Ruer and Shütz	[11]	1910	.50	do		1,502	do	Ni-1,451	1,507
Loebe and Becker	[12]	1912	.20	do		1,525	do	Ni-1,451	1,530
Ruer and Kaneko	[13]	1913	.16	Porcelain	CO	1,525	do	Ni-1,451	1,529
Ruer and Fick	[14]	1913	.20	do	N_2	1,524	do	Ni-1,451	1,529
Ruer and Klesper	[15]	1914	.20	do	N_2	1,524	do	Ni-1,451	1,529
			.04	do	N_2	1,528	do	Ni-1,451	1,533
Burgess and Waltenberg	[16]	1914	.015 to .03		Vacuo and H_2	1,530	Optical	{Pd-1,549 {Ni-1,452	1,534
Rümelin and Fick	[17]	1915	.04	Porcelain		1,526	Thermocouple	Ni-1,451	1,531
Ruer and Goerens	[18]	1917	.04	do	N_2	1,528	do	Ni-1,451	1,533
Hanson and Freeman	[19]	1923	.13	Alumina	N_2	1,530	do	Ni-1,452	1,534
Tritton and Hanson	[20]	1924	>.08	Porcelain	N_2 and A	1,535	do	Pd-1,555	1,534
W. P. Sykes	[21]	1926	.025	Alundum	H_2	1,532	Optical and thermocouple.	Ni-no value	
Andrew and Binnie	[22]	1929	(1)	do	Cracked ammonia	1,537	Thermocouple	Extrapolated above 1,200° C	
Jenkins and Gaylor	[23]	1930	.02 to .10	Alumina	H_2	1,527	Optical	Pd-1,555	1,526
Chipman and Marshall	[24]	1940	.031	Beryllia support	Moist H_2	1,535	Thermocouple	Pd-1,555	1,534
Present work		1940	<0.01	Beryllia	He	1,539	Optical	Au-1,063	1,539

[1] Decarburized Armco.

Most of the values for the melting point of iron were determined with thermocouples. The difference between the iron and palladium points is so small, about 20° C, that this difference will be very nearly the same on practically any temperature scale. The difference between the iron and nickel points, however, is large enough to make the extrapolation from one point to the other depend significantly upon the emf-temperature relation used. The values obtained with thermocouples calibrated with nickel, therefore, are subject to an additional uncertainty not present in those values determined with thermocouples checked with freezing or melting palladium.

The values generally accepted for the melting points of pure nickel and pure palladium on the International Temperature Scale are 1,455° and 1,554° C, respectively. Although the assumption that the various investigators had available nickel or palladium as pure as the metals used in establishing these points is very doubtful, the values reported for the melting point of iron have been adjusted on that assumption, as shown in the last column of table 1. This adjustment brings the values into much better agreement, reducing the spread from 53° to 22° C and the average deviation from 10° to 5° C, and probably adjusts most of the reported values in the right direction—but the uncertainties involved in the adjustment should be kept in mind.

Three determinations in which relatively pure iron was used and in which the temperature scale employed is definitely stated deserve special consideration. These are the determinations of Burgess and Waltenberg [16], Jenkins and Gaylor [23], and Chipman and Marshall [24].

Burgess and Waltenberg reported a result of 1,530° C as the mean of two values by different methods, 1,533° C by the micropyrometer method in which the iron was heated on a platinum strip in hydrogen and 1,528° C by melting the iron in an Arsem furnace both in vacuo and in hydrogen. We attribute the lower value obtained in the Arsem furnace to carbon contamination from the furnace atmosphere and to poor blackbody conditions. Considering only the micropyrometer value, the adjusted value of Burgess and Waltenberg becomes 1,537° C.

Jenkins and Gaylor used several methods, including a crucible method with partially immersed blackbodies. They obtained a value of 1,527° C and reported that the presence of iron vapor interfered with their observations. In our opinion, the low value they obtained is not surprising in view of the arrangement of their blackbodies.

Chipman and Marshall suspended strips of carbonyl iron in hydrogen and melted them in a uniformly heated enclosure with the measuring junction of a thermocouple near the specimen of iron. The melting temperature was taken as the temperature at which drops of metal fell from the specimen. The value 1,535° C reported apparently applies for iron in equilibrium with hydrogen, inasmuch as the iron was heated very slowly as the melting point was approached. Part of the difference of 4° C between this value and the value obtained in the present work is undoubtedly due to hydrogen, and it is also probable that the iron was slightly contaminated by silicon due to the reduction of the silica in the porcelain tube which was close to the sample. On the whole, however, their result and

the micropyrometer result of Burgess and Waltenberg are the two values to which the most weight should be given.

III. PRESENT WORK

1. MATERIALS

The materials used in this work consisted of 2 samples designated as "high-purity" iron, 1 sample of slightly lower purity than the above, 1 sample of Armco ingot iron, and 19 steels.

The high-purity iron was prepared by Thompson and Cleaves [1] by reducing purified iron oxide to sponge iron, melting the sponge, and remelting first in hydrogen and then in vacuo. The samples used were taken from the ingots designated in their paper as numbers 3 and 15 and have been numbered to correspond with the ingots from which they were taken. These ingots were examined spectroscopically and by chemical methods for the presence of 55 elements. The impurities detected were chiefly sulfur, oxygen, and silicon, with lesser amounts of carbon, phosphorus, copper, hydrogen, and nitrogen. The total of impurities detected was less than 0.01 percent. The complete analyses of the ingots are given in the paper by Thompson and Cleaves. All of the impurities detected are listed in table 4.

The sample of iron identified as Y in table 4 came to us indirectly from the Westinghouse Research Laboratory in 1934. The analysis of this sample as reported by that laboratory is given in table 4.

The samples of Armco ingot iron and the 19 steels were machined from bars which had been used for the preparation of the Standard Samples corresponding to the designations given in table 4. The analyses, with the exception of the oxygen content of Sample 55, are those given in the February 18, 1935 Supplement to National Bureau of Standards Circular C398.

No analyses were made of the samples after the freezing-point determinations.

The temperature coefficient of electrical resistance of relatively pure materials will serve as a criterion of purity after a correlation between the two has been established. To provide data for such correlation for iron, we have given, in table 2, corresponding values for some of the samples used in this work. However, unless the wires are given essentially the same heat treatment before the measurements, differences in physical condition may mask the differences due to composition.

The values in table 2 are the coefficients for the intervals 0° to 100° C, $(R_{100}-R_0)/100R_0$, measured on wires 0.02 inch in diameter which had been heated in vacuo for 2 hours at 800° C.

TABLE 2.—*Corresponding values of temperature coefficient of resistance and amount of impurities in iron*

Sample	$\frac{R_{100}-R_0}{100R_0}$	Impurities	
		Weight percent	Atomic percent
3 and 15	0.00652	<0.010	<0.028
Y	.00644	<.028	<.081
Ingot iron	.00590	.18	.32

2. GENERAL PROCEDURE

The procedure followed was, in a general way, similar to that employed by the authors in determining the freezing temperatures of several of the platinum metals [25, 26]. The sample of iron or steel, about 75 grams in weight, was machined from an ingot or rod to the size and shape required to fit the crucible and an axial hole was drilled to accommodate a sight tube. The sample, together with the refractory blackbody sight tube, was placed in a refractory oxide crucible, as shown in figure 1 or 2. This entire assembly was then placed inside a Pyrex glass enclosure in which a desired atmosphere could be maintained. The metal was heated by means of a high-frequency induction furnace and the temperature measured by means of a precision optical pyrometer sighted into the blackbody.

In any experiment of this nature, every effort should be made to select a crucible material and atmosphere which will not alter the chemical composition of the metal during heating at temperatures above its melting point and to arrange the apparatus so that the atmosphere admitted to the enclosure will surround the metal and not be significantly contaminated by adsorbed gases released by the refractory during heating.

3. CRUCIBLES

At the time the freezing-temperature measurements were made on steels, accounts of investigations with iron and steel indicated that these materials could be melted in aluminum oxide crucibles without any appreciable contamination from the crucible. Consequently, aluminum oxide crucibles were used for all the steels except two. In these two cases beryllium oxide was used, not because it was considered superior to aluminum oxide, but merely to gain some information regarding its suitability for use with pure iron. Subsequently, Thompson and Cleaves reported that beryllium oxide was preferable to aluminum or magnesium oxide for melting pure iron. Beryllium oxide crucibles were therefore used exclusively in the later work on pure iron.

The beryllium oxide crucibles and accessory parts were prepared by the general method described by Swanger and Caldwell [27]. Pure unfused beryllium oxide was heated to 1,800° C in a graphite container by means of a high-frequency induction furnace. The calcined oxide was then heated to about 1,100° C in oxygen to remove any carbon either combined or in discrete particles, and was then broken up and ground in a steel ball mill to pass a 200-mesh screen. The finely divided material was treated with HCl to remove iron and then washed with distilled water. The crucibles were formed by tamping the material moistened with a solution of beryllium chloride (4 g of $BeCl_2$ in 100 ml of water) into a graphite mold lined with a layer of paper. The interior of the crucible was formed by drilling with a properly shaped tool while the material was still moist. The mold containing the crucible was heated in air to about 1,100° C for 2 hours. This enabled the crucible to be slipped out of the mold. It was then placed on a tungsten sheet inside a graphite container and fired to 1,800° C by means of an induction furnace.

The crucible cover and the cone above it were made separately in the same way except that before finally firing to 1,800° C, they were

cemented together with a paste made from beryllium oxide and the beryllium chloride solution. The blackbody sight tubes were formed by rolling a small amount of the paste on a brass rod, as described by Swanger and Caldwell. The sight tubes were also fired to 1,800° C.

The aluminum oxide crucibles and accessory parts were made in the same way, with the following exceptions: (1) The material was commercially available crystalline alumina which was prepared for tamping by grinding in a porcelain ball mill; and (2) aluminum chloride was used as a binder.

4. ATMOSPHERE

By a process of elimination, helium was selected as the atmosphere in which to melt both the irons and steels. Atmospheres containing oxygen or carbon were immediately rejected because both of these elements are readily taken up by iron. According to Smithells [28], nitrogen at a pressure of 1 atmosphere is soluble in liquid iron at 1,540° C to the extent of about 0.03 percent by weight, and hydrogen at the same pressure is soluble in liquid iron at the melting point to the extent of about 0.0023 percent and in solid iron at the melting point to the extent of about 0.0011 percent by weight. These percentages may appear small, but according to Van't Hoff's law of the freezing-point lowering, 0.0023 percent of hydrogen (by weight) will lower the freezing point of iron about 3° C. Our experience with the volatility of metals and refractories at high temperatures led us to expect very little success in obtaining an accurate measurement of the freezing temperature of iron in vacuo.

Helium was selected as the atmosphere in which to measure the freezing temperature, because all the available evidence indicates that the solubility of helium in either iron or steel is considerably less than that of nitrogen or hydrogen.

For comparison purposes the freezing temperature of high-purity iron was also measured in an atmosphere of hydrogen and in vacuo. The value obtained for the freezing temperature in hydrogen was lower than that in helium, indicating that helium was less soluble than hydrogen. The value obtained in vacuo is lower than that in either helium or hydrogen. This is attributed to the absorption of light by iron vapor present in the line of sight.

The helium used was sufficiently freed of oxygen to prevent any noticeable oxidation of the samples. Commercial helium was passed through copper chips at about 700° C to remove oxygen and then through magnesium perchlorate and phosphorus pentoxide to remove water vapor. The hydrogen used was passed through the same system.

5. ARRANGEMENT OF APPARATUS

The samples of pure iron were mounted as shown in figure 1, and the samples of steel, for greater convenience in changing samples, were mounted as shown in figure 2. In the first case we were primarily interested in obtaining accurate values for the freezing temperatures of a few samples of pure iron, whereas in the latter case we were interested in obtaining values for the initial freezing temperatures of a large number of steels and were willing to make some sacrifice of accuracy for the sake of convenience.

For the pure iron, figure 1, the sample was insulated on all sides

to promote temperature uniformity in the metal. Large temperature differences at the time of freezing must be avoided if an accurate measurement of the freezing temperature is to be obtained. The insulating material immediately surrounding the crucible will prevent the excessive loss of metal if, by chance, any cracks should develop in the crucible during repeated heating and cooling. The comparatively coarse insulating material permits the ready passage of gas.

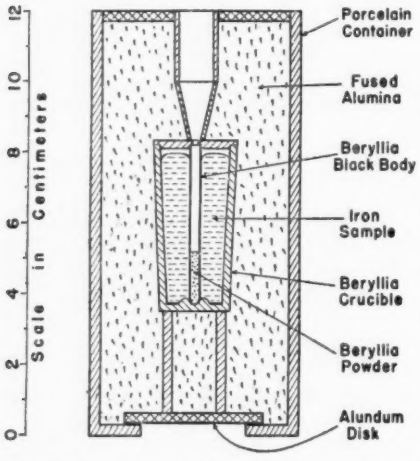

FIGURE 1.—*Cross section showing method of mounting iron samples for the measurement of the freezing temperature.*

In the mounting for the steels, figure 2, any one sample could be easily replaced by another. Unfused aluminum oxide was used for the insulation because it is a better thermal insulator than the fused material. The unfused oxide might also have been used for the pure iron except for the fact that the particles are much smaller and pack more closely, making it difficult to pass a gas through it to the melting-point sample. In all of these measurements no difficulty of any nature was encountered with either type of crucible.

The Pyrex enclosure in which the samples were heated in the desired atmospheres is shown in figure 3. The mica diaphragms, with central openings to permit sighting into the blackbody, reduce the convection currents. The Pyrex tube was closed with a Pyrex plate sealed to the tube with Cementyte. The cemented joint was cooled by a number of small air jets. Thin sheets of mica between the porcelain container and the Pyrex tube restricted the flow of gas around the porcelain container. The alundum disk in the bottom of the porcelain container permitted the gas, admitted to the top of the tube, to flow through the porcelain container. Before heating a sample, the gas (helium or hydrogen) was passed slowly through the enclosure for approximately

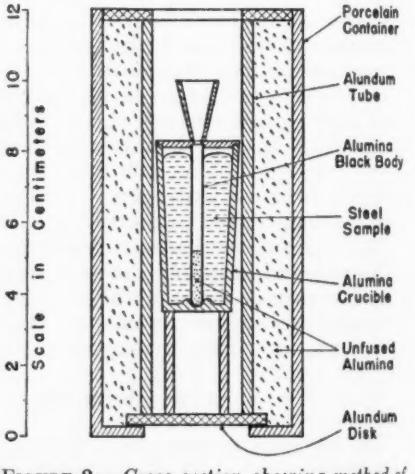

FIGURE 2.—*Cross section showing method of mounting steel samples for the measurement of the freezing temperature.*

16 hours. The sample was then heated slowly so that the gases released from the refractory during heating would be carried off.

The Pyrex tube fitted closely inside of the water-cooled coil of the induction furnace.

6. TEMPERATURE MEASUREMENTS

The optical pyrometer used was the one designed by Fairchild and Hoover [29] and has been briefly described elsewhere [25]. It was fitted with a 45° total-reflecting prism for sighting into the blackbody.

The temperature measurements are expressed on the International Temperature Scale of 1927, in which

$$\log_e \frac{J_2}{J_1} = \frac{C_2}{\lambda}\left(\frac{1}{1336} - \frac{1}{t+273}\right),$$

where J_2/J_1 is the ratio of brightness at the wavelength, λ, of blackbodies at temperature t° C and at the gold point (1,336° K), and C_2 is a constant equal to 1.432 cm-degrees. In these measurements the effective wavelength, λ, of the red screen in the optical pyrometer was 0.6532 μ. For the measurements on high-purity iron, the brightness ratio, J_2/J_1, was measured by utilizing a sector disk of such an opening that it reduced the brightness of the blackbody at the freezing temperature of iron to very nearly that of a blackbody at the gold point. The transmission of the sector disk used was determined on a circle dividing engine as 0.01406 with an uncertainty of 0.00002. An uncertainty of 0.00002 in the transmission corresponds to about 0.25° C in the determination of the freezing temperature of iron.

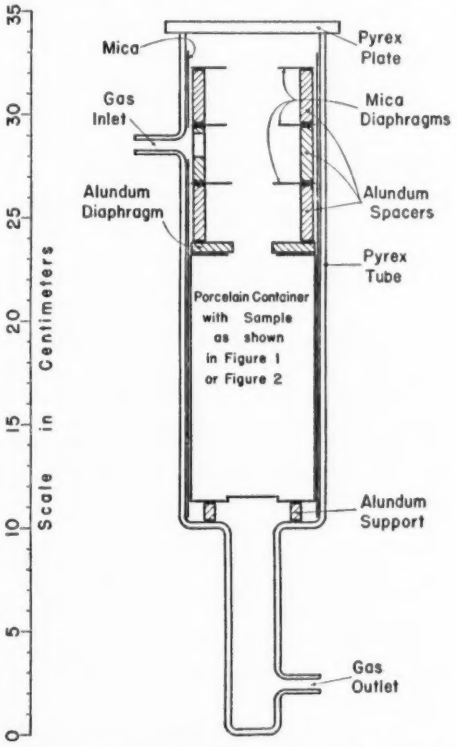

FIGURE 3.—*Assembly used for heating samples in a controlled atmosphere.*

For the observation on the blackbody at the gold point, an assembly of the same dimensions as that shown in figure 1 was used. The only difference was that for the gold, thorium oxide refractories were used.

For the measurements on the steels, an absorption glass was used instead of a sector disk.

IV. RESULTS

The results obtained on the two samples of high-purity iron in helium and in hydrogen are given in table 3. Each freeze represents the mean of from 12 to 24 photometric settings. During the course of these measurements, approximately the same number of observations was taken on the blackbody at the gold point.

TABLE 3.—*Summary of observations on the freezing temperature of high-purity iron*

Ingot number	Observer	Atmosphere			
		Helium		Hydrogen	
		Freezing point	Number of freezes	Freezing point	Number of freezes
		°C		°C	
3	H. T. W.	1538.8	4	1537.5	4
3	W. F. R.	1539.1	5	1538.3	7
15	H. T. W.	1539.1	3	1537.7	3
15	W. F. R.	1538.6	3	1538.0	6
Mean		1538.9		1537.9	

The difference of 1° C between the freezing point of iron in helium and in hydrogen is attributed to solution of hydrogen in iron. This difference of 1° C was checked by arranging an experiment in which either hydrogen or helium could be admitted to the enclosure by means of a two-way stopcock, and taking a series of freezes with the iron in hydrogen, then in helium, and finally shifting back to hydrogen.

The measurements in vacuo yielded a result about 3° C below that obtained in helium. This difference is attributed to the absorption of light by iron vapor. The 3° C corresponds to an absorption of light of about 2 percent. The effect of pressure, as such, upon the freezing temperature of iron is only a small fraction of a degree per atmosphere.

The final values obtained in helium for the initial freezing temperature of the high-purity irons, and other irons, and the steels are given in table 4.

In the measurements on some of the steels, there was a decided drift upward in the values obtained for successive freezes. The maximum drift observed was 4° C per freeze for Sample 12d. This was attributed to changes in the chemical composition. In such cases, the value reported is that obtained by plotting the values for the various freezes as a function of time of heating and extrapolating the curve back to zero time.

It is recognized that, in general, a steel does not freeze completely at any one temperature but instead has a freezing range. In a few cases we were able to detect the lower limit of the freezing range, but these were exceptional. However, no difficulty was encountered in establishing the temperature of initial freezing.

TABLE 4.—*Chemical analyses and initial freezing temperatures of irons and steels*

Sample	Percentage, by weight, of—																		Initial freezing temperature	
	C	Mn	P	S	Si	Cu	Ni	Cr	V	Mo	As	Sn	Al	W	Co	O_2	H_2	N_2	Measured	Computed[c]
																			°C	°C
Pure irons:																				
15	(a)	nil	(b)	0.0013	nil	<0.002	nil	nil	nil	nil	nil	nil	<0.001	nil	nil	0.0019	0.0002	0.0006	1,538.9	.....
3	0.001	nil	(b)	.0026	0.003	<.002	nil	nil	nil	nil	nil	nil	nil	nil	nil	.0005	.0001	.0004	1,538.9	.....
Y	<.005	<0.002	<0.002	<.005	<.004	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<.01	.....	.....	1,537.4	>1,537.5
Ingot iron:																				
55	.013	.019	.003	.017	.001	.041	0.020	0.002	.....	0.002	0.012	0.008	0.002	.....	0.006	.03	.....	.005	1,534.5	1,534
Steels:																				
15b	.101	.559	.032	.038	.292	.145	.083	.064	0.005	.003	.008	.....	.....	.....	.....	.....	.....	.....	1,527	1,524
8d	.078	.482	.099	.083	.018	.013	.004	.007	.003	.003	.007	.....	.....	.....	.....	.....	.....	.....	1,525	1,526
11d	.202	.430	.006	.041	.027	.010	.008	.008	.002	.001	.008	.....	.....	.....	.....	.....	.....	.....	1,520	1,522
111	.202	.662	.023	.020	.292	.122	1.75	.272	.003	.215	.016	.....	.....	.....	.....	.....	.....	.....	1,513	1,510
72	.294	.651	.016	.020	.137	.064	0.288	.911	.012	.149	.012	.....	.....	0.045	.....	.....	.....	.....	1,509	1,511
106	.343	.484	.020	.020	.250	.142	.129	1.29	.008	.164	.009	.....	1.06	.....	.....	.....	.....	.009	1,507	1,506
12d	.418	.344	.013	.036	.016	.015	.007	0.015	.002	.002	.008	.....	.....	.....	.....	.....	.....	.....	1,506	1,508
32b	.413	.624	.016	.018	.217	.117	1.21	.638	.006	.005	.017	.....	.....	.....	.....	.....	.....	.....	1,496	1,499
73	.314	.276	.023	.031	.360	.033	0.072	13.93	.034	.005	.011	.....	.....	.....	.....	.....	.....	.....	1,494	1,491
30c	.489	.707	.019	.014	.237	.099	.080	0.977	.235	.010	.016	.....	.....	.....	.....	.....	.....	.....	1,494	1,497
33b	.366	.700	.037	.032	.233	.114	3.48	.029	.005	.003	.016	.....	.....	.....	.....	.....	.....	.....	1,493	1,492
21c	.574	.630	.062	.030	.107	.050	0.152	.166	.007	.005	.008	.....	.....	.....	.....	.....	.....	.....	1,484	1,492
22b	.674	.935	.084	.042	.123	.009	.003	.003	.005	.002	.004	.0004	.....	.....	.....	.....	.....	.....	1,483	1,484
100	.617	.39	.023	.022	.192	.123	.151	.180	.011	.005	.....	.....	.....	.....	.....	.....	.....	.....	1,481	1,486
14b	.817	.493	.008	.031	.009	.017	.074	.018	.....	.008	.012	.018	.....	.....	.....	.....	.....	.....	1,477	1,478
101	.061	.554	.011	.013	.763	.056	8.44	17.56	.044	.006	.....	.005	.....	.....	.....	.....	.....	.028	1,464	1,462
50a	.660	.287	.020	.007	.48	.047	0.045	3.52	.970	.009	.042	.026	.....	18.25	.....	.....	.....	.....	1,460	1,461
35a	1.03	.345	.037	.036	.387	.267	.254	0.264	.011	.004	.009	.....	0.005	.....	.....	.....	.....	.....	1,456	1,457
51	1.29	.271	.011	.014	.250	.044	.02	.02	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1,434	1,442

[a] This lot of iron was not analyzed for carbon. The carbon content of other lots of iron prepared by the same methods was 0.001 percent or less.
[b] These lots of iron were not analyzed for phosphorus. The phosphorus content of other lots of iron prepared by the same methods was less than 0.0005 percent.
[c] The values in this column were computed by summing up the effects of the individual elements.

V. EFFECT OF IMPURITIES AND ALLOYING ELEMENTS

The effect of certain individual elements upon the initial freezing temperature of iron can be obtained from phase-equilibrium diagrams, but these give no information as to the effect produced when two or more foreign elements are present in the iron. The effect of certain elements, individually or in combination, upon the freezing temperature of iron can be calculated, on certain assumptions regarding dilute solutions, from Van't Hoff's law of the freezing-point lowering; but, in many of the cases, the assumptions regarding dilute solutions do not hold even for very small percentages of the foreign elements. Consequently the effect of several elements upon the freezing temperature, when all are present at the same time, can only be found from experiments.

The measurements of the initial freezing temperatures of the steels, and the chemical analyses, provide data for establishing the effects of various impurities and alloying elements on the freezing temperature of iron when several of the elements are present at the same time. Our results indicate that, in general, when a number of other elements are present in iron, the effect of any one is the same as it would be if the other elements were not present and that the total effect of all the elements is the sum of the effects of the elements taken separately.

The lowering of the freezing temperature of iron by the individual impurities and alloying elements usually present in steels is given in table 5. These values for the individual elements, other than silicon, tungsten, and the gases, were obtained from phase-equilibrium diagrams, chiefly from the International Critical Tables. It was found that the sum of the individual freezing-point depressions could be made more nearly equal to the observed depressions by reducing the value obtained from the phase-equilibrium diagram for silicon from 14° to 8° C per percent and by making the freezing-point depression for tungsten equal to 1° C per percent. The value for tungsten is based upon the observation on only one sample, 50a, and consequently does not apply generally. The values for the gases were computed by using Van't Hoff's law for the freezing-point depression. The values for the freezing-point depressions in table 5 have been found to apply within reasonable limits for amounts of the elements within the ranges given in the last column of table 5.

The initial freezing temperatures of the various irons and steels computed from the chemical analyses and the lowering effects of the individual elements are given in the last column of table 4. The agreement of the temperatures calculated by use of table 5 with the observed temperatures is very good, the average difference being less than 3° C. In general, the computed values are slightly higher than the observed values. However, the amount of the gases present in the steels was determined only in a few instances, and in these instances the agreement between the observed and computed freezing temperatures is exceptionally good. If the gases present in the other steels had been determined and taken into consideration, the computed values would have been lowered and consequently, in most cases, brought into better agreement with the observed values.

This method of computing the initial freezing temperature from the analysis cannot be expected to hold for percentages of impurities or alloying elements outside of the range in which it has been verified by

experiment. Table 6 shows how well it holds for cast irons. The analyses and experimentally determined initial freezing temperatures were taken from a paper by Ash and Saeger [30]. The initial freezing temperatures, computed by adding the effects of the separate elements, are given in the last column. With the exception of the values for samples XIII and XIV, the average difference between the observed and computed values is 5° C.

TABLE 5.—*Depression of the initial freezing (liquidus) temperature of iron per percent by weight for each of the impurities or alloying elements usually found in steels, and the amounts of the various elements in the materials investigated*

Element	Depression of freezing point per percent by weight	Range of elements in materials investigated (percentage by weight)
Hydrogen	1,300° C (computed)	0 to ?
Nitrogen	90° C (computed)	0 to 0.03
Oxygen	80° C (computed)	0 to 0.03
Carbon	Varies as follows: 65° C at 0% 70° C at 1% 75° C at 2% 80° C at 2.5% 85° C at 3% 91° C at 3.5% 100° C at 4%	0 to 3.8
Phosphorus	30° C	0 to 0.7
Sulfur	25° C	0 to 0.08
Arsenic	14° C	0 to 0.5
Tin	10° C	0 to 0.03
Silicon	8° C	0 to 3
Manganese	5° C	0 to 1.5
Copper	5° C	0 to 0.3
Nickel	4° C	0 to 9
Molybdenum	2° C	0 to 0.3
Vanadium	2° C	0 to 1
Chromium	1.5° C	0 to 18
Aluminum	0	0 to 1
Tungsten	1° C	18% W with 0.66% C

TABLE 6.—*Chemical analyses and initial freezing temperatures of cast irons*

Sample number	Percentage, by weight, of—					Initial freezing temperature	
	C	Mn	P	S	Si	Measured	Computed
						° C	° C
XI	1.96	0.25	0.14	0.06	0.85	1,380	1,378
X	2.00	.24	.15	.06	.84	1,380	1,375
XII	1.97	.27	.14	.06	1.50	1,365	1,372
VI	2.29	.35	.27	.04	1.24	1,350	1,341
III	3.08	.44	.35	.04	1.68	1,250	1,249
IV	3.10	.48	.35	.04	1.69	1,250	1,245
XIV	2.89	.44	.66	.03	2.88	1,195	1,251
XIII	3.27	.52	.59	.03	2.87	1,155	1,207
IX	3.60	.58	.67	.03	2.86	1,150	1,157
VIII	3.63	.59	.68	.03	2.87	1,150	1,154
VII	3.76	.54	.46	.05	2.10	1,150	1,147

We hesitate to suggest that a value computed from the data in table 5 is more accurate than an experimentally determined value, but it is difficult to see why sample XIV with 2.89 percent of carbon should melt 55° C lower than sample IV with 3.10 percent of carbon. The other constituents are about the same with the exception of

silicon, which according to the observations on other samples has an effect of only 8° C per percent. In the case of samples XIII and IX, the compositions are practically identical except for the carbon content, and it is difficult to believe that 0.33 percent of carbon would lower the freezing temperature by only 5° C. The discrepancy between the observed and computed values for samples XIII and XIV may be due to the presence of some impurity that was not sought for in the analysis, since in both cases the measured value is lower than the calculated one.

VI. DISCUSSION OF RESULTS

The mean of the observations on the freezing point of the high-purity iron in helium, 1,539° C, is believed to be accurate to ±1° C. The recognized sources of error and the magnitude of the uncertainty introduced in the final result by each, are estimated to be as follows: (1) Transmission of sector disk, 0.25° C; (2) effective wavelength of red screen, 0.3° C; (3) photometric matching, 0.25° C; and (4) departure from blackbody conditions, 0.2° C.

If the impurities in the samples of high-purity iron were all present in the maximum amounts reported and if helium is not soluble in iron, the freezing point of absolutely pure iron would not be more than 0.5° C higher than that of the high-purity irons used in this investigation.

The mean of the observations on the freezing point of the high-purity iron in hydrogen, 1,538° C, is somewhat less accurate than the value in helium, because it is subject to all the uncertainties listed above in addition to the uncertainty in the amount of absorbed hydrogen at the beginning of freezing. According to Smithells [28], the amount of hydrogen, at a pressure of 1 atmosphere, absorbed by iron increases from about 0.0001 percent, by weight, at 400° C to about 0.0011 percent in the solid at the melting point and to about 0.0023 percent in the liquid at the melting point. These are equilibrium values which require some time to establish. In our experiments the iron was heated only about 20° C above the melting point and was maintained molten for only a few minutes before freezing. It is very doubtful that the iron absorbed the equilibrium amount of hydrogen in these few minutes. Our experience with silver in air [31] would indicate that only a small percentage of the difference between 0.0011 and 0.0023 percent would be absorbed under these conditions.

The value of 1,536° C obtained for the freezing point of the high-purity iron in vacuo does not mean that the iron was freezing at 1,536° C. It was evident from the deposits of iron on the inner surfaces of the cone directly above the opening in the blackbody that iron vapor had been present in the light path. Jenkins and Gaylor reported seeing clouds of iron vapor in their experiments and went to considerable trouble to devise methods to eliminate them. The difference between 1,539° and 1,536° C can be accounted for by an absorption of light of 2 percent. No "clouds" of vapor were detected in our experiments.

The freezing temperature of the iron identified as Y was measured in the same way as the high-purity irons, and the result obtained should be just as accurate. The value obtained for the initial freezing

temperature of the Armco ingot iron is the result of 10 freezes and should be accurate to ±2° C. Each of the values for the initial freezing temperature of the steels is the result of from three to five freezes and should be accurate to ±3° C.

The method of computing the initial freezing temperatures of the steels from the chemical analyses and the lowering of the freezing point by the separate elements is entirely empirical and should be used with discretion. However, this method seems to hold reasonably well for considerable ranges of composition.

VII. SUMMARY

1. The freezing temperature of high-purity iron in helium at atmospheric pressure is 1,539°±1° C.

2. The freezing temperature of high-purity iron is 1° to 3° C lower in hydrogen than in helium.

3. The freezing temperature of Armco ingot iron in helium is 1,534°±2° C.

4. The depression of the initial freezing temperature of an iron or steel by most of the elements commonly found in steels is independent of the presence of other elements.

5. The initial freezing temperature of a steel can be calculated from the chemical composition.

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WASHINGTON, November 5, 1940.

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RESEARCH PAPER RP1376

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ELECTROPHORETIC STUDIES OF NYLON

By Milton Harris and Arnold M. Sookne [1]

ABSTRACT

The surface electrical properties of "unoriented" and of highly "oriented" nylon fibers were investigated by a microelectrophoretic technique. Both types of fibers exhibited definite reversals of charge but at different pH values—the unoriented fiber at pH 3.9 and the oriented at pH 2.7. Since nylon consists of hydrocarbon chains linked together by amide groups, it appears that reversal of charge is principally dependent on the presence of the amide groups, especially since hydrocarbons do not exhibit reversal of sign of charge under comparable conditions. This suggests that amide groups may also influence the electrophoretic behavior of other substances, such as proteins. The difference in the electrophoretic behavior of the two types of nylon is attributed to the difference in their degrees of "crystallinity."

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I. INTRODUCTION

The development of new synthetic textile fibers frequently makes it necessary to alter common practices in the textile industry. Thus, new fibers may require new types of scouring, dyeing, or finishing agents depending on the nature of the material. A well-known example of this was the necessity of developing entirely new types of dyestuffs to dye cellulose acetate fibers. That the development of nylon has also presented new problems is not surprising since the chemical and physical properties of this fiber are very different from those of any of the other textile fibers. A number of these problems, such as those involving finishing treatments, are dependent in part on the nature of the surface of the fibers, and more especially on their surface electrical properties. Accordingly, an investigation was undertaken of the surface electrical properties of nylon by the microelectrophoretic technique as previously used in this laboratory for studying similar characteristics of wool [1],[2] cotton [2], and silk fibers [3].

In addition to these considerations, it appeared that such an investigation might be of value in the general problem of interpretation of electrophoretic data of protein materials. While it has long been recognized that the net charge of proteins under ordinary condi-

[1] Research Associates at the National Bureau of Standards, representing the Textile Foundation.
[2] Figures in brackets indicate the literature references at the end of this paper.

tions depends primarily on the degree of ionization of acidic and basic groups (the content of COO— and NH_3^+ groups), it has more recently been recognized that other groups may play a part as well [4]. Of such groups, the amide groups are present to a large extent in both the proteins and nylon. In the latter, however, they are practically the only polar groups and their role should be more readily interpretable than in the proteins, where many different polar groups are present.

II. MATERIALS AND METHODS

Two types of materials were used. One consisted of the so-called "undrawn" or "unoriented" fibers which are obtained by extruding the molten polyamide from a spinneret. The second type of material was the "drawn" or "oriented" fibers obtained by stretching the unoriented fibers approximately fourfold in length.

Both of these materials were extracted with ether to remove surface greases. The method of reducing fibers to a convenient particle size for electrophoretic measurements, the preparation of the suspensions, and the method of measuring the electric mobilities of the suspended particles have been described elsewhere [1, 2, 3].

All measurements were made in buffer solutions of 0.02 *M* ionic strength. In the pH range between 2.0 and 2.8, hydrochloric-acid-potassium-chloride solutions were used. Above pH 2.8, acetic-acid-sodium-acetate buffers were used.

III. EXPERIMENTS AND DISCUSSION

Figure 1 shows the mobility as a function of pH for samples of the oriented and unoriented nylon fibers. The oriented nylon exhibits a reversal of sign of charge at about pH 2.7 and the unoriented material at about pH 3.9.

Nylon is a polyamide prepared by the interaction of a diamine and a dicarboxylic acid and has the following type of structure:

$$-CO-R-CO-NH-R'-NH-CO-R-CO-NH-R'-NH,$$

where R and R′ may represent 4 and 6 methylene ($-CH_2-$) groups. Chemically, therefore, nylon consists principally of hydrocarbon chains linked together by amide groups. Presumably the ends of the chains may contain free basic or acidic groups, but the presence of such groups in a polymer of this type may be very difficult to detect [5]. Regardless of this, however, the proportion of these groups which may be expected to be free in the nylon polymer is extremely small and should not appreciably influence the behavior of the material. It appears then, that the reversal of sign of charge may be principally dependent on the presence of the amide groups, especially since a number of hydrocarbons already studied [6] have been shown not to exhibit reversal of sign of charge under comparable conditions.[3] A similar result was also noted earlier in this laboratory [7] in investigating the electrophoretic behavior of dibenzyldiketopiperazine. This

$$\begin{array}{l} C_6H_5-CH_2-CH-CO-NH \\ \qquad\qquad\quad | \qquad\quad | \\ \qquad\qquad NH-CO-CH-CH_2-C_6H_5. \end{array}$$

[3] The sign of charge of these substances can undoubtedly be reversed in solutions of much lower pH than are used in this work and than are generally used in similar investigations on proteins. It should also be noted that substances which do not show reversals of sign of charge in the presence of uni-univalent salts frequently do so when polyvalent ions are present.

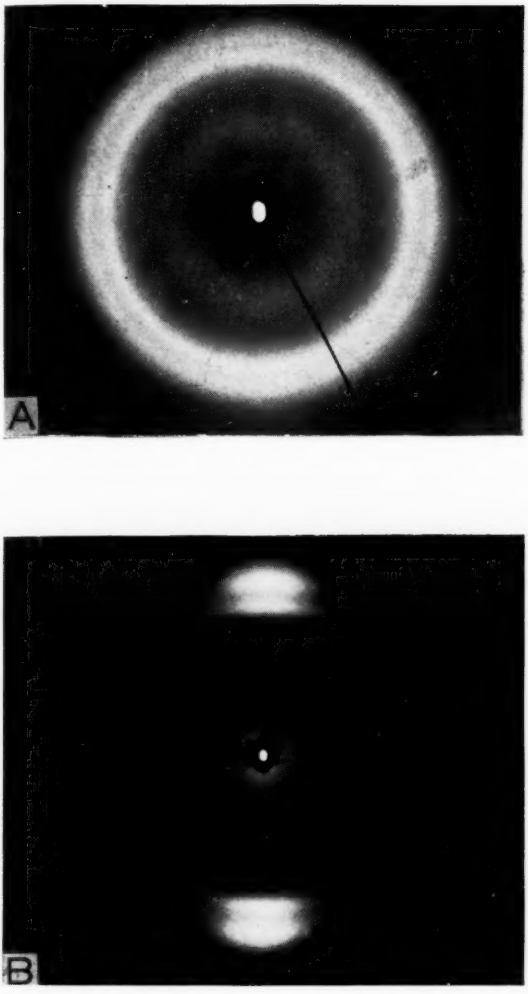

FIGURE 2.—*X-ray diffraction patterns of nylon.*
A, unstretched; *B*, stretched.

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substance, which is not amphoteric in the usual sense (in that it contains no free carboxyl or amino groups) but contains two amide groups, exhibited a definite reversal of charge at pH 2.9. Thus the possible influence of the amide groups on the electrophoretic behavior of some substances, such as proteins, must be given consideration, although such influence may be obscured when large numbers of acidic and basic groups are present.

That different pH-mobility curves and different isoelectric points were obtained for the oriented and unoriented nylons is of considerable interest, although it was not unexpected. Similar differences have been noted for the electrophoretic properties of other substances when

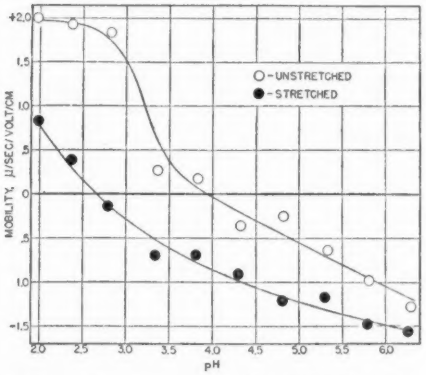

FIGURE 1.—pH-*mobility curves for stretched and unstretched nylon.*

studied in both the amorphous and crystalline states. Earlier work by Wintersteiner and Abramson [8] showed that the isolectric point of amorphous insulin was at pH 5.35, while that of insulin crystals was found at pH 5.0.[4] It was further shown by Abramson and Moyer [4] that the isoelectric points of various amino acid crystals all occur at pH values below the values found for the amorphous or dissolved substances. Although it has not been common practice to consider fibers crystalline in this same sense, nevertheless, the X-ray diffraction patterns do indicate a far more orderly or "crystalline" arrangement in the oriented nylon, as shown in figure 2. Stretching the unoriented nylon probably produces a fairly parallel arrangement of the long-chain molecures. Such a process tends toward a maximum of interaction between the molecules, and thus changes the character of the surface. This would be expected to alter the electrophoretic mobility of the substance. On the basis of these considerations, it may be concluded that the difference in the electrophoretic behavior of the two types of nylon used in this investigation is related to the difference in their degrees of "crystallinity."[5]

[4] It was not known in this case how much adsorbed insulin was on the surface of the crystalline insulin. The difference might have been greater if no dissolved insulin had been present.

[5] A similar phenomenon was recently reported by Kanamaru and Takada [9], who found that the ζ-potential of viscose varies with the velocity at which the fibers were originally spun—i. e., with their degree of parallelism.

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RELATIVE AFFINITIES OF THE ANIONS OF STRONG ACIDS FOR WOOL PROTEIN [1]

By Jacinto Steinhardt, Charles H. Fugitt, and Milton Harris [2]

ABSTRACT

Evidence presented in previous papers supported the view that wool immersed in solutions containing hydrochloric acid combines stoichiometrically not only with the hydrogen ions of the acid but with chloride ions as well. As a consequence it appeared that the specific affinities for wool of the anions of different acids might vary considerably, and that therefore the positions of the titration curves of this protein with respect to the pH axis might vary by correspondingly large amounts according to the acid used.

The present paper describes measurements of the combination of wool with 19 different acids, ranging in complexity from some of the mineral acids most commonly used through the simpler aromatic sulfonic, carboxylic, and phenolic acids to a soluble monoazo acid dye. It is shown that wide differences exist between the positions with respect to the pH axis of the titration curves of wool obtained with different strong acids, and that these differences may be ascribed to wide variations in the anion dissociation constants characterizing the corresponding protein-anion combinations. Equations previously derived to account for effects caused by variations in chloride concentration have been generalized for use in calculating these dissociation constants. A scale of relative affinities of anions for wool, based on these constants, and applicable to acid dyes, is proposed. Predictions as to the effects of variations of anion concentration and of temperature, based on the same generalized equations, have been tested and confirmed.

Measurements of the combination of a number of the same acids with a soluble protein, crystalline egg albumin, have also been made. Since qualitatively similar differences in the positions, with respect to the pH axis, of the titration curves obtained with different acids are found with both proteins, it is concluded that the property of combining with anions is not restricted to insoluble proteins. The affinity of anions for proteins of both classes appears to increase with the dimensions of the anion, and is higher in aromatic than in aliphatic ions of the same size. The bearing of these relationships on the well known specific effects of ions on proteins and on the nature of the forces involved in the binding of anions by proteins is considered.

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[1] Part of the material in the present paper was presented at the meeting of the American Society of Biological Chemists at New Orleans, La., in March 1940 [37].

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I. INTRODUCTION

It is well recognized that the fastness of wool dyes to washing, and their related properties of exhausting onto wool or other protein fibers from a dye bath, vary widely not only among different classes of dyes, but also among the members of a given class. These variations are frequently regarded as manifestations of differences in affinity of the various dyestuffs for the fibers. The existence of similar differences in the affinities of ions for proteins in general is suggested by the frequently reported and repeatedly investigated instances of specific differences in the effects of various ions on protein behavior.

A possible basis for understanding such specific effects in terms of the participation of the anions of acids and the cations of bases in the combination of wool protein with acid and base has already been described in two earlier communications [36, 38].[3] Evidence was presented in support of the view that the combination of wool with hydrochloric acid may be resolved into two partial reactions, one with hydrogen ions and one with chloride ions, and that each reaction is governed by its own equilibrium constant. On the basis of this assumption it proved possible to account for the large effects produced by varying the concentration of potassium chloride on the combination of wool with hydrochloric acid. Among other consequences of this view was a strong possibility that the specific affinities for wool of the anions of different acids might vary considerably, and that therefore the positions of the titration curves of this and other proteins with respect to the pH axis might vary by correspondingly large amounts according to the acid used. The existence of such a variation would constitute critical evidence in support of the hypothesis of anion association, since alternative analyses of the effect of salt, based upon the Donnan membrane-equilibrium equations [36], offer no obvious basis for the prediction of such an effect.

The present paper describes measurements of the combination of wool with 19 different acids, ranging in complexity from some of the mineral acids most commonly used in the laboratory through the simpler aromatic, sulfonic, carboxylic, and phenolic acids to a soluble monoazo acid dye. The acids employed were selected from among those readily available, with the purpose in view of obtaining data which could be simply calculated and interpreted in terms of molecular size, chemical structure, and the effects of specific substituents. For this reason most of the results reported in the present paper deal with acids which are monobasic and virtually totally dissociated in water. A limited number of weak acids which are analogues of some of the

[3] Figures in brackets indicate the literature references at the end of this paper.

strong acids used (for example, benzoic acid is analogous with benzenesulfonic acid in the sense used here) have been included for the specific purpose of examining the effects of this analogy. An effort has also been made to include organic acids with all of the more common dissociating groups. Since strong acids containing carboxylic or phenolic radicals as dissociating groups can be obtained only by loading with substituents such as halogens or nitro groups, other related acids have been included in order to make possible an independent appraisal of the effect of various substituent groups, and of more strictly physical factors, such as the size and shape of the anion produced.

In order to determine whether differences in affinity between the ions of different acids are restricted to wool, or to the insoluble class of proteins for which the theory of anion association was specifically proposed [36], measurements have also been made of the combination of a number of the same acids with a well-characterized *soluble* protein, crystalline egg albumin. Demonstration that the hypothesis of anion association is applicable to both would have an obvious bearing on the interpretation of the titration curves of both classes of proteins, and of the wide range of phenomena which depend on the combination of protein with acid or base.

II. EXPERIMENTAL PROCEDURE

Details of the purification of the wool and of the method used in measuring pH values and the amounts of acid combined have already been described [36, 38]. To improve the accuracy of estimation of quantities depending on pH differences, pH values are given to three decimal figures wherever the precision of the measurements, determined as previously described, is better than 0.003 unit. The absolute accuracy of the values given depends on the accuracy of the pH values assigned to the primary standards [36].

Additional details which concern the materials used in the present investigation and modifications of the earlier procedures are described in the following subsections.

1. MATERIALS

(a) WOOL

Except where otherwise noted, all of the measurements were made with wool from the batch used in a previous investigation [38]. Samples of this wool, on incineration at 700° C, yielded a residue of 0.26 percent by weight; the hydrogen-ion equivalence of their cation content, as shown by electrodialysis [32], was 0.036 millimole per gram. Determination by the methods previously described [36] showed that of this ash 0.032 millimole per gram was combined as base, and that therefore a correspondingly larger quantity of acid is neutralized by this wool than would be neutralized by ash-free fibers.

In calculating the results obtained with most of the acids used, this small excess of acid has been subtracted from the apparent amounts of acid bound at every pH. The validity of this procedure was self-evident in the measurements with hydrochloric acid, but it is somewhat open to question when anions of much higher affinity for wool than that of chloride are present. It is shown elsewhere in

this paper that the affinities of certain anions for wool are so high as to produce a net negative charge on the fibers even in acid solutions in which combination with hydrogen ions usually results in a net positive charge. Since the negative charge may be neutralized in part by cations other than hydrogen ion, including the cations naturally in the ash, not all of the alkaline ash is available for neutralization of the dissolved acid. No correction for ash has been applied therefore to the data obtained with Orange II, the anion of which has the highest affinity of those included in this paper. With picric and flavianic acids, next highest in order of affinity, the full correction has been applied; a slight overcorrection may result, especially in the range of pH in which only small amounts of acid are bound.

(b) PREPARATION OF EGG ALBUMIN

Crystalline egg albumin was prepared by the method of Kekwick and Cannan [22], recrystallized five times, and dialyzed free of sulfate. The resulting stock solution contained 7.02 mg of nitrogen per milliliter and gave a dry residue, on evaporation in a vacuum oven at 100° C, of 45.2 mg/ml. The closeness of the pH of the diluted stock solution (5.09 at 22° C) to the isoionic pH of this protein, indicates that the preparation was practically free of combined ions. The nitrogen content, 15.5 percent, is in good agreement with the values hitherto reported.

(c) SOURCE AND PURIFICATION OF ACIDS

Care was required in the removal of ionogenic impurities from all the acids used, especially when the affinity of the anion of the acid for the protein was much lower than the affinity of one or more of the anions contributed by the impurities. When only a small proportion of all the acid present is combined (as at low pH), the amount combined may be almost entirely the impurity and not the acid under investigation.

In every case the absence of sulfate or chloride was established by analysis. The possibility of the presence of appreciable amounts of salts was excluded by titrating weighed portions of the dry preparations.

The source and method of purification of the acids used are itemized below:

1. Hydrochloric, nitric, and trichloroacetic acids were commercial cp products used without further purification.

2. Hydrobromic acid was a commercial cp product, from which appreciable quantities of free bromine were removed under vacuum.

3. Ethylsulfuric acid was prepared by dissolving diethyl sulfate in a large excess of absolute ethanol, and heating the solution to 55° C for 24 hours, as described in Beilstein [I, 325]. The solution was then evaporated at room temperature under vacuum, and dilutions were made of the resulting approximately 1 *M* stock solution. The solutions of this acid thus contained varying but small quantities of ethyl alcohol. Analyses showed the presence of small amounts of sulfate ion, which were removed by the addition of an accurately determined equivalent quantity of barium hydroxide. The methods of preparation used for both this acid and the acid which follows excluded the possibility of the presence of salts.

4. Benzenesulfonic acid was prepared by treating redistilled benzenesulfonyl chloride with silver oxide and passing hydrogen sulfide through a solution of the resulting silver salt.

5. *o*-Nitrobenzenesulfonic acid, 2,4,6-trichlorophenol, 2,4-dinitrophenol, and 2,4,6-trinitroresorcinol were Eastman products purified by two or more recrystallizations from water.

6. *p*-Toluenesulfonic acid, 2,4-dinitrobenzenesulfonic acid, *o*-xylene-*p*-sulfonic acid, naphthalene-β-sulfonic acid, and flavianic acid (1-naphthol-2,4-dinitro-7-sulfonic acid) were Eastman products from which traces of impurities which were only slightly soluble in water were removed by filtration.

7. Picric acid (2,4,6-trinitrophenol) was Merck's U.S.P. grade containing 15 percent of added water, used without further purification.

8. Benzoic acid was a National Bureau of Standards Standard Sample (No. 39e).

9. 2,5-Dichlorobenzenesulfonic acid and 4-nitrochlorobenzene-2-sulfonic acid were Eastman products which contained appreciable amounts of sulfuric acid as an impurity. They were purified by precipitating the sulfate with an accurately determined equivalent quantity of barium hydroxide.

10. The sodium salt of Orange II (*p*-sulfobenzene-azo-β-napthol), obtained from the Eastman Kodak Co., was further purified by repeated salting out according to the method of Robinson and Mills [27]. The free acid was prepared by dissolving the salt in 2 *M* hydrochloric acid and washing the precipitated dye acid with several portions of the same hydrochloric acid solution. When appreciably higher or lower concentrations of the acid are used, the loss of dissolved acid dye in subsequent washing is very considerable. The precipitate of acid dye was air-dried, then oven-dried at 60° C, and finally dried to constant weight in a vacuum desiccator over KOH. Tests showed that hydrochloric acid had been completely removed in the drying process. The acid dye, as well as its sodium salt, is very soluble in water, but efforts to prepare concentrated solutions of the acid revealed the presence of a small quantity of a less soluble impurity. Most of the impurity was removed by decanting concentrated solutions from the insoluble residue.

2. METHODS

(a) MEASUREMENT OF ACID COMBINED WITH WOOL

Strong acids which gave colored solutions were titrated with bromthymol blue, instead of the bromcresol purple previously used, because its color change was more easily detected. With weak acids thymol blue or phenolphthalein was used. Densely colored solutions of Orange II were titrated to an electrometric end point at pH 7.

Although it proved possible in the experiments with hydrochloric acid to attain equilibrium within a period of 72 hours at 0° C, and in still shorter periods of time at 25° C, and 50° C [36, 38], some of the acids used in the present investigation required very much longer periods of time at all these temperatures. Thus, with flavianic acid a period of 23 days at 0° C proved insufficient for the combination of over 0.42 millimole per gram, while values approaching the usual

maximum of about 0.8 millimole per gram were obtained in a few days at 25° C. A period of 30 days at the latter temperature proved insufficient with Orange II, while at 50° C a period of 5 days sufficed. When the attainment of equilibrium requires long periods, the rate of uptake may become undetectably small when much less than equilibrium amounts have been combined. In general, the length of time which must be allowed for essentially complete attainment of equilibrium increases with the affinity for wool of the anion of the acid.

The reversibility of the combination with several of the acids used has been demonstrated by subjecting the fibers to prolonged washing and by replacement of combined anions on immersing the fibers in solutions of other anions having higher affinities.

(b) MEASUREMENT OF ACID COMBINED WITH EGG ALBUMIN

The acid combined by a dissolved protein obviously cannot be measured by the change in the titratable acidity of a solution, as was possible with wool. Methods of calculating this quantity for dissolved proteins are all based on differences in pH between solutions of the acid alone and of the acid plus protein. They have been described by Loeb [25], Cohn, Green and Blanchard [8], Kekwik and Cannan [22], and Hitchcock [20], among others, and are summarized in reviews by Hitchcock [21] and by Schmidt [30]. In the present investigation pH measurements were made of serial dilutions of each acid used in the absence of protein as well as in the presence of 0.113 percent by weight of dissolved egg albumin. The difference in pH caused by the protein gives directly the proportion of the total acid present which has been neutralized (combined by protein), if it can be assumed that the presence of this low concentration of protein is without significant effect on the activity of the uncombined acid. Differences in the activity coefficients of the acid due to its dilution by partial neutralization were taken into account when the initial concentration of acid was so high as to warrant application of the correction. However, owing to the low concentration of the protein, total concentrations of acid in excess of 0.01 M gave rise to such small pH differences that calculations of bound acid at pH values below 2 were subject to large uncertainties. In general, the effect of the variation of activity coefficients with concentration is considerably smaller than these uncertainties.

In calculating the results obtained with flavianic acid, it was assumed that every molecule of the acid contributed two hydrogen ions to the solution. A comparison of the pH values with the titers of the experimental solutions containing no protein indicated that this assumption is approximately valid in the pH range of the curve for this acid. Any error introduced by the assumption probably affects only the highest two points on the curve, which would be slightly lower if an exact calculation were made.

III. RESULTS AND DISCUSSION

1. RESULTS OBTAINED IN THE ABSENCE OF SALTS

The results obtained with 18 different acids in the absence of salt at 0° C are summarized in table 1. The data obtained with the 16 strong acids included are assembled in figure 1. In drawing the curves, an effort has been made to represent the measurements fairly

with smooth curves rather than to preserve the appearance of homogeneity of the data. The resulting tendency of certain neighboring curves to cross or converge is usually within the range of effects which may be ascribed to experimental error.

The most noticeable feature of the data in figure 1 is the wide range of positions with respect to the pH coordinates which the titration

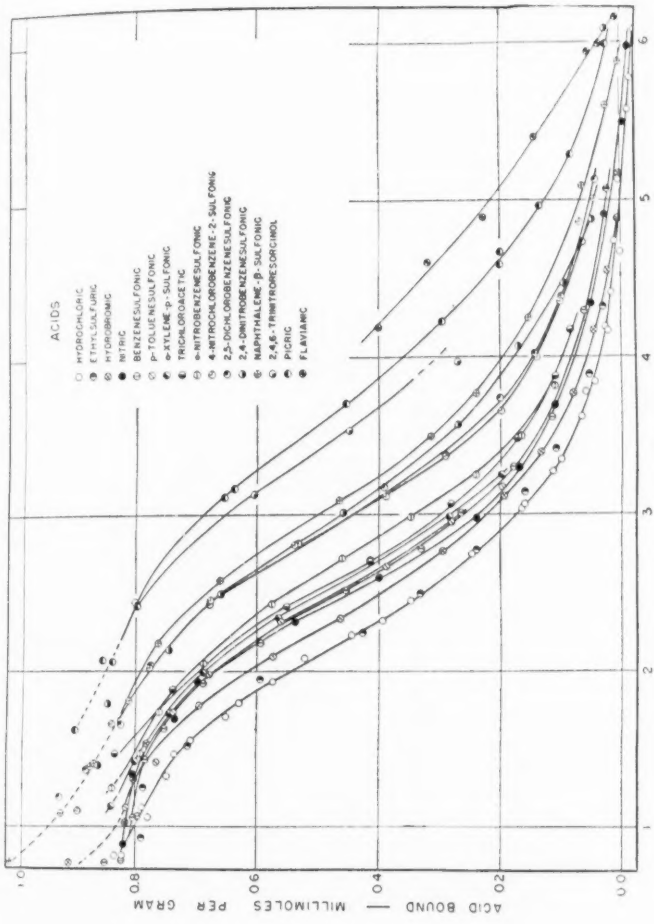

FIGURE 1.—*Combination of wool protein with 16 different strong acids as a function of* pH *at 0° C.*

The measurements with flavianic acid are represented in milliequivalents instead of millimoles. Points representing the combination of less than 0.14 millimole per gram of three acids (*p*-toluenesulfonic, *o*-xylene-*p*-sulfonic, and *o*-nitrobenzenesulfonic) would fall at lower pH values than are required for coincidence with the curves for the other acids, and have been omitted to avoid confusing the figure. Data for the two dibasic acids, flavianic and trinitroresorcinol, are only partially represented, as explained elsewhere.

curves occupy. It is also apparent that except for minor deviations the curves form a coherent family, near neighbors following a fairly parallel course. The change in the slopes of the lower portions of the curves, from the more sharply inflected curve for hydrochloric and ethylsulfuric acids at one end of the pH range to the less sharply inflected curves for picric and flavianic acids at the other end, tends to be gradual and progressive. The appearance of the curves as a group is entirely consistent with the view that they all describe analogous phenomena.

TABLE 1.—*Combination of wool protein with various acids at 0° C, in the absence of added salt*

Hydrochloric acid	
pH	*Millimoles/g*
0.447	0.868
.635	.838
.813	.836
.822	.812
1.062	.779
1.125	.789
1.327	.748
1.470	.735
1.553	.708
1.711	.652
1.797	.629
1.931	.574
2.085	.522
2.228	.445
2.320	.394
2.451	.348
2.748	.247
3.039	.166
3.072	.160
3.272	.114
3.348	.101
3.620	.067
3.783	.060
3.844	.045
4.169	.022
4.198	.027
4.414	.017
4.668	.001
4.740	.012
5.140	.004
5.58	[a]−.012
5.79	[a]−.018
6.1	[a]−.023

Hydrobromic acid[b]	
0.783	0.875
1.024	.791
1.308	.785
1.539	.775
1.780	.687
2.087	.566
2.332	.456
2.771	.288
3.115	.187
3.394	.127
3.772	.074
4.168	.039
4.550	.011
4.878	[a]−.003
5.170	[a]−.010

Nitric acid	
0.601	0.900
.884	.820
1.340	.805
1.697	.735
1.936	.697
2.311	.532
2.594	.399
2.978	.239
3.296	.170
3.694	.111
4.339	.050
4.906	.027
5.50	[a]−.006
5.97	[a]−.012
6.6	[a]−.026

Ethylsulfuric acid	
0.928	0.790
1.252	.788
1.520	.713

Ethylsulfuric acid	
pH	*Millimoles/g*
1.949	0.595
2.242	.427
2.498	.332
2.792	.240
3.140	.151
3.418	.104
3.890	.054
4.320	.031

Trichloroacetic acid	
1.474	0.836
1.883	.738
2.415	.552
2.700	.414
3.070	.281
3.481	.173
3.890	.109
4.170	.079

Benzenesulfonic acid	
0.772	0.853
.791	.825
1.060	.804
1.069	.796
1.435	.783
1.632	.751
1.915	.689
2.181	.594
2.512	.454
2.786	.331
3.010	.265
3.615	.115
4.295	.061
5.072	.023

o-Nitrobenzene-sulfonic acid	
1.248	0.838
1.473	.791
1.739	.760
2.058	.689
2.434	.575
2.718	.460
2.989	.347
3.253	.242
3.502	.166
3.812	.111
4.405	.041
4.752	.012
4.930	.003
5.428	[a]−.005

p-Toluene-sulfonic acid	
1.124	0.817
1.411	.764
1.732	.738
1.984	.678
2.315	.560
2.667	.388
2.954	.280
3.178	.198
3.398	.131
3.638	.085
3.997	.036
4.280	.013

2,4-Dinitroben-zenesulfonic acid	
pH	*Millimoles/g*
1.399	0.863
1.787	.848
2.138	.746
2.490	.657
3.012	.457
3.570	.270
4.069	.171
4.470	.094
4.872	.048
6.1	.028
6.4	[a]−.010

o-Xylene-*p*-sulfonic acid	
1.132	0.840
1.422	.800
1.734	.743
1.995	.688
2.344	.564
2.716	.415
2.994	.284
3.254	.199
3.445	.130
3.754	.085
4.044	.036
4.335	.009

[b] 2,5-Dichloroben-zene-sulfonic acid	
1.191	0.926
1.372	.875
1.663	.819
2.044	.770
2.436	.670
2.825	.525
3.153	.385
3.385	.285
3.743	.195
4.020	.136
4.393	.092
4.732	.059
5.132	.036

[b] 4-Nitrochloro-benzene-2-sulfonic acid	
1.201	0.882
1.384	.868
1.663	.833
2.026	.770
2.453	.670
2.806	.526
3.110	.381
3.368	.285
3.653	.192
3.999	.132
4.352	.094
4.859	.063
5.001	.038

Picric acid	
1.624	0.904
2.059	.838
2.072	.857
2.414	.797
3.116	.654
3.165	.638
3.708	.453
4.229	.296

Picric acid	
pH	*Millimoles/g*
4.586	0.200
4.666	.200
4.964	.134
5.296	.082
5.826	.042
6.00	.023
6.35	.015

[b] 2,4,6-Trinitro-resorcinol	
2.442	0.794
3.130	.598
3.540	.441
3.917	.264
4.204	.176
4.387	.112
4.627	.076
4.897	.049
5.310	.030
5.460	.016

Napthalene-β-sulfonic acid	
0.769	1.028
1.084	0.930
1.404	.871
1.806	.810
2.178	.762
2.598	.660
3.094	.464
3.494	.314
3.771	.240
4.244	.153
5.091	.064
5.606	.024
5.90	.002

Flavianic acid	
	[c] *M-eq/g*
4.191	0.400
4.601	.318
4.898	.227
5.410	.142
5.95	.054
6.10	.034
6.26	.010

Benzoic acid	
	Millimoles/g
3.534	0.168
3.710	.124
3.900	.082
4.191	.062
4.398	.036
4.842	.021
4.989	.005

2,4-Dinitrophenol	
4.83	0.085
5.27	.056
5.63	.031
5.90	.011
6.19	[a]−.004
6.33	[a]−.011

[a] Negative values indicate loss of bound acid or combination with hydroxyl ion.
[b] A different batch of wool having an exceptionally low ash content and a slightly higher maximum combining capacity was used in these measurements.
[c] Note that in this group milliequivalents rather than millimoles are given.

It is a familiar fact that the position on a pH grid of the titration curves of the common weak bases is determined by their own dissociation constants and not by the dissociation constant or by other chemical properties of the acid employed in the titration. Sets of titration curves obtained with any number of weak or strong acids would be expected to be practically superimposed on one another. Exceptions arise, as in the work of Cannan and Kibrick [7] and of Greenwald [16, 17] with carboxylic acids and phosphates, when the compound formed by the reaction of the base with certain acids is not typically salt-like and does not exist in solution predominantly in the dissociated form. These exceptions find their counterparts, in the case of ampholytes, in the formation of only partially dissociated stoichiometric complexes between the anions of the acids and the ampholyte which has combined with hydrogen ions. When these are formed, the positions of the titration curves and to a lesser extent their shapes, will differ according to the extent to which the anion is dissociated [36].

The wide range of the measurements serves to emphasize the very large differences which may be found when different acids are used. If the position of each curve is characterized by the pH value at which half the maximum amount of acid (about 0.4 millimole per gram) is taken up, there is a difference of almost 2 pH units between the curves shown at the extremes of the series. Between these extremes the results obtained with the other acids are distributed with a fair degree of uniformity, with no evident tendency to resemble closely the results obtained with hydrochloric acid, the acid which has hitherto been most widely used in measuring the acid-combining properties of proteins.[4]

Attention must also be directed to another feature of many of the data represented in figure 1. This is the combination of amounts of acid in excess of 0.82 millimole per gram, the amount earlier reported as the "maximum" obtained with hydrochloric acid. The maximum of 0.82 millimole per gram was sometimes exceeded by small amounts when high concentrations of acid (>0.2 *M*) were used. Measurements of acid combined are susceptible to a relatively large experimental error when such high concentrations of acid are used, because (a) only a small proportion of the acid initially present is combined, (b) some acid hydrolysis of the protein cannot be avoided, and (c) exchanges of water between the partially hydrated fibers and the solutions, difficult to evaluate exactly, have a relatively large influence on the results obtained. For these reasons little weight was placed on these measurements, although they were consistent with the results of similar determinations on bone and hide collagen [2], and might be explained by the presence in proteins of extremely feeble basic groups, such as the amide groups of glutamine and asparagine, and the large content of peptide nitrogen. Special interest, therefore, attaches to the finding that in every case in which sufficiently high concentrations of other acids are used, a second step in the curve of acid combination apparently begins at pH values more acid than those at which the curve that covers the usually investigated range of combination flattens. Since this fairly well-marked "maximum" of about 0.82 millimole per gram has been shown to correspond closely to the

[4] Comparable differences between the titration curves of wool with hydrochloric and with picric acids were found by Elöd [11].

primary amino content of the fibers, the additional uptake in more concentrated solutions must be accounted for by the more weakly basic groups or by combination by means of entirely different mechanisms. One such alternative mechanism is suggested by the titration curve of wool with Orange II at 50° C, shown in figure 6, in which the excess combination is even larger. The molecules of many dyes are known to be aggregated in aqueous solutions to a greater or lesser extent [40]. Although no evidence suggesting that Orange II is highly aggregated has ever been put forward, the failure of its solutions to obey Beer's law suggests that some degree of aggregation may occur in all but extremely dilute solutions of this dye. Combination of the fiber, at least in part, with aggregated dye anions might then account for the large amounts taken up, and for the anomalous relation to pH of the amounts combined at high concentrations.

The displacement of the pH range, in which the excess combination with most of the acids is found, removes much of the uncertainty attending the earlier observation of similar excess combination with hydrochloric acid. All three of the possible sources of experimental error mentioned above are reduced or eliminated when the excess combination occurs in the presence of low concentrations of acid. In addition, the excess found in concentrated solutions ($1.03 - 0.82 = 0.21$ in the case of naphthalenesulfonic acid) is far outside the limits of experimental error or of the possible range of effects on concentration due to exchange of water between fibers and solution.

Although the scatter of the points representing the combination of more than 0.8 millimole per gram is considerable, the portions of the curves which represent the second step of acid combination appear to run parallel to one another to about the same extent as do the main curves at higher pH values. The displacements of these parts of the curves thus appear to correspond, qualitatively at least, to the similar displacements of the parts of the curves which represent the combination of the first 0.82 millimole per gram. Should further work establish that the concepts applied in this paper to the lower section of the curves may also be employed in the analysis of the excess combination of acid, the possibility is opened of studying in an aqueous environment the combination of these weakly basic groups with acid, and thus determining the number of these groups and the extent of their dissociation.

2. CALCULATION OF THE AFFINITY OF ANIONS FOR WOOL

Any simple analysis of the experimental results in terms of the hypothesis of stoichiometric anion association depends on the assumption that the curve of acid combination is homogeneous, that is, the curve is determined by the combination of hydrogen ions and anions by sets of essentially similar groups in the fiber. If this is not the case, it is meaningless to ascribe shifts in the positions of the titration curves as a whole to variations in a single anion dissociation constant presumed to characterize each protein-anion combination. The assumption is empirically justified when the displacement in the positions of the curves affects all parts of the curve to the same extent. With the minor exceptions noted elsewhere, the data in figure 1 appear to justify a treatment based upon such homogeneity.

(a) RELATION OF THE POSITIONS OF THE TITRATION CURVES TO THE ANION AFFINITY

Since it has been assumed that differences in the positions of the curves with respect to the pH axis are determined by differences in K_A', the protein-anion dissociation constant, it is appropriate to attempt to evaluate K_A' for the anions of each of the acids used by means of the position of the corresponding titration curve. The reciprocal of K_A' is referred to hereafter as the anion affinity constant, or simply as the anion affinity.

It has already been shown [36] that changes in the positions of the titration curves obtained with a single acid, which are produced by adding different amounts of the anion of the acid in the form of neutral salts, can be described by the equation:

Fraction of wool combined

$$\equiv\frac{[WHA]+[WH^+]}{[WHA]+[WH^+]+[WA^-]+[W^\pm]}=\frac{1}{1+\frac{K_H'}{a_H}\left(\frac{a_A+K_A'}{a_A+K_A}\right)} \quad (1)$$

in which a_H and a_A represent the activities of the hydrogen ion and of the anion respectively, the terms containing W represent ionic states of wool, and the constants K'_H, K'_A, and K_A govern the following hypothetical dissociation equilibria:

$$K_H' \qquad WHA\rightleftharpoons WA^-+H^+ \quad (2)$$
$$K_A' \qquad WA^-\rightleftharpoons W^\pm+A^- \quad (3)$$
$$K_A \qquad WHA\rightleftharpoons WH^++A^-. \quad (4)$$

The constant K_H governing a fourth possible equilibrium:

$$WH^+\rightleftharpoons W^\pm+H^+ \quad (5)$$

does not appear in eq 1, since it is obvious that the four constants are interrelated by the relation $K_H'\,K_A'=K_HK_A$.

The assumptions entering into the definition set down in the left-hand part of eq 1 have already been discussed [36]. It has also been shown that each experimental curve is actually somewhat broader (covering a wider range of pH values) than the S-shaped curves described by eq 1, but that the displacement between curves obtained at different values of a_A are accurately described by the equation.[5] The difference between the curves obtained with and without a constant concentration of anions present is also accurately described by converting eq 1 into the equivalent form, valid when salt is absent:

Fraction combined

$$\equiv\frac{[WHA]+[WH^+]}{[WHA]+[WH^+]+[WA^-]+[W^\pm]}=\frac{1}{1+\frac{K_H'}{a_H}\left(\frac{a_H+K_A'}{a_H+K_A}\right)} \quad (6)$$

The derivation of eq 1 and 6 made clear that their validity is limited to conditions under which the term $[WH^+]$ is greater than the term

[5] Reasons have been given for attributing this broadening of the experimental curves to the high degree of polyvalency of the wool protein [36], which has the effect of introducing an empirical exponent on the hydrogen-ion term in the simple equation [23]. The simplified treatment also necessarily leaves out of account all effects due to the mutual interaction of dissociating groups in the molecule except for the effect implied broadly in the inequalities $K_H'<K_H$ and $K_A<K_A'$.

$[WA^-]$. These conditions are realized, except when much salt is present, when K_A' is greater than K_H. The latter constant has not been evaluated, but it must be considerably larger than K_H', which has been assigned the value 6.3×10^{-5} at 0°C [36]. Thus eq 6 cannot be used for anions having affinities which result in a displacement of the titration curve by more than 1 or 2 pH units to the right of the position of the curve obtained with hydrochloric acid.

It is possible to derive an equation, similar to eq 6, which has the complementary condition for validity, that is, $[WA^-]>[WH^+]$, or $K_A'<K_H$. This equation, applicable to acids with high affinity for wool, is obtained by equating the fraction combined to

$$\frac{[WHA]+[WA^-]}{[WHA]+[WA^-]+[WH^+]+[W^\pm]}$$

instead of to

$$\frac{[WHA]+[WH^+]}{[WHA]+[WH^+]+[WA^-]+[W^\pm]}$$

and leads to the general result:[6]

$$\text{Fraction combined}=\frac{1}{1+\frac{K_A}{a_A}\left(\frac{a_H+K_H}{a_H+K_H'}\right)} \tag{7}$$

or, in the absence of salt, to the equivalent form:

$$\text{Fraction combined}=\frac{1}{1+\frac{K_A}{a_H}\left(\frac{a_H+K_H}{a_H+K_H'}\right)} \tag{8}$$

The dependence of the pH coordinate of the midpoint of each curve on K_A' or K_A, in terms of either eq 6 or 8, may be described by equating the right-hand member of each equation to 0.5. When this is done with eq 6, the result is

$$K_A'=\frac{a_H(a_H+K_H)}{K_H'}-a_H=\frac{a_H^2-a_HK_H'}{K_H'}-\frac{K_H}{K_H-a_H}. \tag{6'}$$

With eq 8 the result is

$$K_A'=\frac{K_H}{K_H'}-a_H\left(\frac{a_H+K_H'}{a_H+K_H}\right). \tag{8'}$$

Equations 6′ and 8′ both contain constants which have not previously been evaluated (K_A and K_H), as well as the constant K_H' to which the value 6.3×10^{-5} has previously been assigned.[7] Because of electrostatic requirements, however, $K_H>K_H'$ and $K_A'>K_A$ [36]. The ratios between pairs of dissociation constants which differ mainly because of the electrostatic effect of a single charge (such as the first and second dissociation constants of symmetrical dicarboxylic acids) always lie between 10 and 1000. If this analogy is accepted, K_H probably lies between $10^{-3.2}$ and $10^{-1.2}$. Since the factor $K_H/(K_H-a_H)$

[6] When salt is present, this equation gives the amount of acid *plus salt* combined since the excess negative charge due to $[WA^-]$ may be neutralized in part by adsorbed cations other than hydrogen ions. The amount of salt combined is large only when anions of high affinity are present, or when high concentrations are used, so that the titration curve falls in a region of high pH values.

[7] In all the calculations that follow, the reasonable assumption is made that K_H' is an intrinsic property of the protein, and is not appreciably affected by the nature of the anion combined ([36], footnote 18).

in eq 6′ must be positive, the curves obtained with acids of lower affinity than hydrochloric acid (not reported in this paper) further restricts K_H to values greater than $10^{-2.1}$. The factor thus approaches unity for values of a_H at the midpoints of practically all the curves represented in figure 1. Thus, between limits represented by the midpoint of the hydrochloric acid curve and the midpoints of curves for which the condition of validity for eq 6′ ($K_A' > K_H$) no longer applies, the equation may be simplified to

$$K_A' \doteq \frac{a_H^2 - K_H' a_H}{K_H}. \qquad (6'')$$

The corresponding simplification of eq 8′, valid when $a_H \ll K_H$ (throughout practically the entire range of pH values in which the midpoints of the curves occur), is

$$K_A' \doteq \frac{a_H^2 + K_H' a_H}{K_H'}. \qquad (8'')$$

The calculation of K_A', the reciprocal of the affinity, thus depends in either case on an accurate knowledge of K_H', the intrinsic acidity constant of the carboxyl groups of the fiber. By assigning the previously determined approximate value at 0° C to this constant, the relation of K_A' to the pH of the midpoint has been calculated for this temperature according to both equations. The result of this calculation is plotted logarithmically in figure 2, in which the midpoint pH, and therefore the value of the affinity constant, according to both eq 6″ and eq 8″ has been indicated for each of the acids represented in figure 1.

It is apparent that in the region of low pH values both equations give practically identical results. The curves approach the same straight line having a slope of 2. In this region the ratio of affinities between two anions is given with a high degree of approximation by the antilogarithm of twice the difference in pH between the midpoints of the titration curves obtained with the acids corresponding to the anions.

At pH values above 3, however, the curves diverge appreciably, and at pH values above 4.2 (pK_H' at 0° C), no physically real values of K_A' are given by eq 6″. Obviously, however, the conditions under which eq 6 and 6″ are valid ($K_A' > K_H$) are no longer realized when the curves are displaced to such high ranges of pH. Since both equations lead to essentially the same relation between K_A' and midpoint pH in the range of pH in which eq 6″ is nearly exact, all calculations of affinity in this paper have been based on eq 8″, regardless of the range of pH in which the midpoint of a given titration curve may lie.

It should be noticed that as the affinity increases, the difference in the positions of the curves produced by a given increase in affinity becomes larger until ΔpH, which at one extreme is equal to $-\frac{1}{2}\Delta pK_A'$, at the other extreme approaches $-\Delta pK_A'$. As the latter limit is approached closely, the affinity constant becomes independent of the value assigned to K_H' and is equal to the midpoint a_H. It will be shown later that the affinities of commercial acid dyes probably fall in this range; thus, the comparison of their affinities relative to one another may be much simplified.

Included in figure 2 is a third curve, also representing eq 8″, in which the value 9.32×10^{-5} has been used for K_H' instead of the

value 6.30×10^{-5}. This curve shows the relation between midpoint pH and log K_A', at a temperature of 25° C [38], and also illustrates the relatively large effect of variations in K_H' on the estimation of K_A' when the latter is large, and the virtual absence of this effect when the latter is small.

By making use of the curve which represents eq 8'' at 0° C, values of K_A' which correspond to each of the acids represented in figure 1 have been determined. These values are given in the fifth column of table 2. The ion with least affinity, chloride, has a value of K_A',

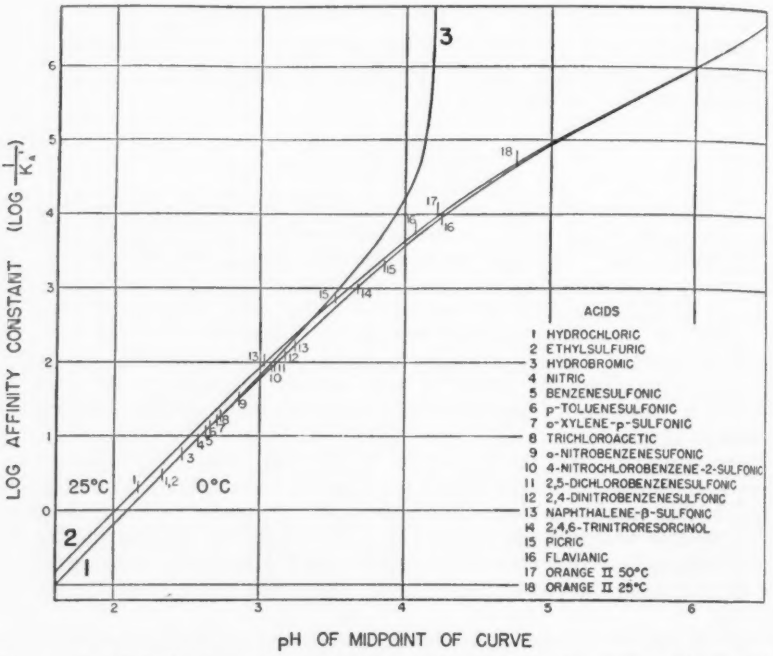

FIGURE 2.—*Relation between the position of the acid titration curve with respect to* pH *and the affinity of the anion of the acid for protein.*

Curves 1 and 2 represent eq 8'' with numerical values of K_H' at 0° and 25° C, respectively, inserted. Curve 3 represents eq 6'' with the numerical value of K_H' at 0° C. Curves 1 and 3 coincide at low pH values.

0.35; picrate, the most tightly bound monovalent ion studied at this temperature, has an affinity almost one thousand times as great as that of chloride, and the divalent flavianate ion has an affinity still higher.[8] It is apparent from the results summarized in table 2 that the affinity of the anion tends to rise as the molecular weight increases but the tendency is not regular and exceptions in the series occur.

Since the experimental curves are broader than those predicted by the much simplified eq 6 and 8, the use of these equations in analyzing the shifts in position of the curves would be rendered more significant if the progressive change in their slopes could also be shown to be a consequence of the same differences in K_A' which result in their displacements to progressively higher pH values. This demonstration

[8] For comparison, the affinity of the divalent flavianate ion is expressed in the same units as the affinities of the monovalent acids in the table.

has been made by substituting representative numerical values in eq 6 and 8. For the sake of definiteness, the value 0.01 has been assigned to the ratio K_H'/K_H, which has been shown to have a range of reasonable values of 0.01 to 0.001. The result of this calculation with six different values of K_A' is shown in figure 3. The calculated curves simulate the curves drawn through the experimental data not only in the relation between the magnitudes of their displacements from one another but also in the relation of their slopes. Thus, the curves representing low values of K_A' (high affinities) are everywhere less steep than those which correspond to high value of K_A' (low affinities). The progressive change in slope at the midpoints of the experimental curves is in the same direction. The qualitative agreement with experiment of these calculations would not be altered by the assignment of other reasonable numerical values to the ratio K_H'/K_H.

TABLE 2.—*Calculation of anion affinity constants* $(1/K_A')$ *at 0° C*

Acid	Molecular weight	pH of midpoint	$-\log K_A'$ from eq 8''	K_A'	Affinity relative to affinity of chloride ion	pH at which curve crosses HCl 0.1 molal curve	Affinity relative to affinity of chloride ion (method of crossing point)
Hydrochloric	36.5	} 2.33	0.45	0.35			
Ethylsulfuric	126.1						
Hydrobromic	80.9	2.47	.74	.18	1.9		
Nitric	63.0	2.58	.96	.11	3.2	1.71	3.9
Benzenesulfonic	158.2	2.63	1.05	.089	3.9	1.71	3.9
p-Toluenesulfonic	172.2	2.66	1.10	.080	4.4	1.73	4.1
o-Xylene-*p*-sulfonic	186.2	2.71	1.21	.062	5.6	1.83	5.2
Trichloroacetic	163.4	2.73	1.25	.056	6.2	1.93	6.6
o-Nitrobenzenesulfonic	203.2	2.86	1.50	.032	11.0	2.05	8.6
4-Nitrochlorobenzene-2-sulfonic	237.6	3.08	1.93	.0118	29.7	2.68	36.8
2,5-Dichlorobenzenesulfonic	227.1	3.09	1.95	.0112	31.2	2.68	36.8
2,4-Dinitrobenzenesulfonic	248.2	3.17	2.09	.0081	43	2.82	51
Naphthalene-β-sulfonic	208.2	3.24	2.23	.0059	59	3.05	86
2,4,6-Trinitroresorcinol	245.1	3.67	3.02	.00096	366		
Picric	229.1	3.86	3.34	.00046	758		
Flavianic	314.2	4.24	[a] 3.94	[a] .000115	[a] 3,020		

[a] Calculated, for comparison, as if the anion were monovalent. Since equilibrium was not attained in 23 days, the relative affinity reported is minimal.

(b) ALTERNATIVE METHOD OF CALCULATING RELATIVE ANION AFFINITIES

In column 6 of table 2 is given the ratio of the affinity of the anion of each acid to that of chloride ion. This has been tabulated because the same relative affinity factor may be calculated directly from the experimental results represented in figure 1 by combining them with the data for hydrochloric acid at constant chloride concentrations in an earlier paper [36]. The values so obtained thus do not depend on the equations just used.

This alternative method of calculation is made possible by the fact that curves obtained in the presence of constant anion concentrations are less steep than those obtained in the absence of salt and therefore cover a wider range of pH values. Thus, for example, a curve representing results obtained with hydrochloric acid at a constant concentration (0.1 *M*) of chloride ions would intersect all of the curves represented in figure 1 with the exception of the curve for flavianic

acid. The intersections with the curves for hydrobromic acid and picric acid would occur so near the extremes of the 0.1 *M* curve that they are not well suited to the present calculation, but all of the other intersections may be used. At the point of intersection of the 0.1 *M* hydrochloric acid curve with any curve in figure 1, the same amounts of two different acids are bound by the wool at the same pH. The same degree of combination with two different anions is thus brought about by the presence of different amounts of the respective anions by virtue of the difference of affinity for wool characterizing these

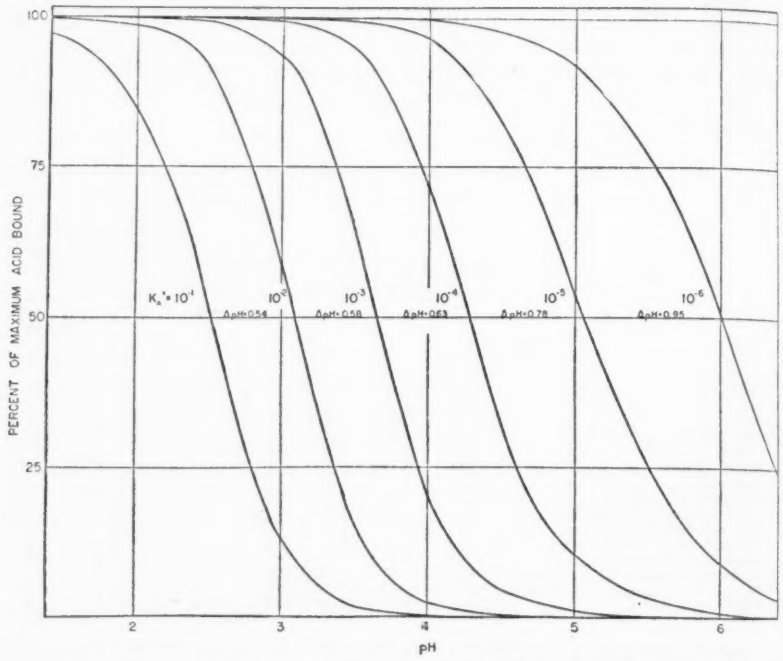

FIGURE 3.—*Dependence of the slope of theoretical titration curves on the protein-anion dissociation constant.*

The two leftmost curves are calculated from eq 6 and the others from eq 8. The change from eq 6 to eq 8 between $K_A'=0.01$ and $K_A'=0.001$ has been made in order to preserve consistency with the assumption $K_B'/K_B=0.01$.

anions. It follows from the range of ratios of K_H'/K_H which has been adopted elsewhere that the ratio of the activities of the two anions in the two different experiments is inversely proportional to the ratio of the respective anion affinity constants. The anion affinity ratios which are obtained should be nearly the same as those given in column 6 of table 2.

The activity of potassium chloride at 0.1 *M* at 0° C is 0.0768 [18]. The assumption has been made that the activity of the anion in each experiment made in the absence of salt is equal to the activity of the hydrogen ion. It is very unlikely that this assumption is true; but the experimental value, pH, is related to the activities of both ions, and methods of obtaining individual ion activities are at present unknown. By dividing 0.0768 by the antilogarithm of the negative

of the pH, the values given in the last column of table 2 have been obtained. In view of the fact that the intersecting curves were drawn freehand and that the two kinds of data were obtained with different lots of wool, the agreement of the values in the last column with those in column 6 is satisfactorily close.[9]

3. RESULTS OBTAINED IN THE PRESENCE OF SALT

It should be possible to predict the displacements that would be found if the same acids were used in the presence of constant concentrations of their anions, added as neutral salts. By starting with eq 1 and 7 instead of eq 6 and 8, and making the same simplification which was carried out in arriving at eq 6'' and 8'', formulas which give the relation between K_A' and the midpoint pH when the measurements are made in the presence of any constant concentration of anions are obtained:

$$K_A' = a_A \frac{a_H - K_H'}{K_H'} \qquad (1'')$$

$$K_A' = a_A \frac{a_H + K_H'}{K_H'} \qquad (7'')$$

In these equations the quadratic term a_H^2, found in eq 6'' and 8'', does not appear. In the range of relatively low affinities, therefore, a given change in K_A' will, in the presence of constant anion concentrations, produce twice the pH change which corresponds to this change in K_A' in the absence of salt. With anions having higher affinities, however, displacements in midpoint pH values obtained in the presence and absence of salt will differ by less than a factor of 2. These predictions have been tested by making measurements with a number of the acids included in table 1, in the presence of 0.1 *M* concentrations of their anions, added as the potassium salts. The results of these titrations are summarized in table 3 and are represented graphically in figure 4. As the figure shows clearly, the difference in the pH values of the midpoints of the hydrochloric acid and benzenesulfonic acid curves is twice as great for curves obtained in the presence of salt as for those obtained in its absence. The difference between the midpoints of the hydrochloric acid and naphthalenesulfonic acid curves is somewhat less than twice as great in the presence of salt as in its absence. Both differences thus agree with the predictions of the equations.

Similar experiments have also been made at 25° C with Orange II in the presence of a constant concentration of its colored anion, added as the sodium salt. The affinity of this anion is so great that it combines with the protein in considerable amounts at pH values at which only small amounts of hydrogen ion are normally taken up. The resulting negative charge on the fiber is neutralized, as in all the cases covered by eq 7 and 8, by adsorption of positive ions. In the presence of salt, appreciable quantities of sodium ions, as well as hydrogen ions, are bound. Thus, eq 7 no longer gives the amount of acid taken up alone, but the sum of the amounts of acid and of salt. Since

[9] A third method of calculating relative affinities has also been made use of. Mixtures of acids have been equilibrated with wool, and the relative amounts of the two acids combined have been determined. The relative affinities of the two anions calculated from this ratio agree closely with the results of the methods described in this paper. Since the method has thus far been applied to experiments with acids for which no data are given in the present paper, and since work with these acids is still in progress, no examples of these calculations are given here.

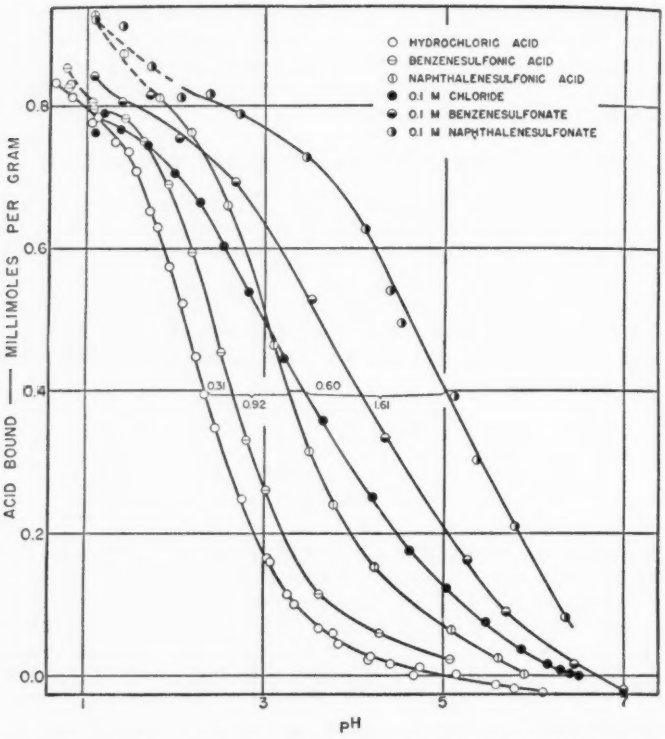

FIGURE 4.—*Comparison of the titration curve of wool protein obtained with three different acids in the absence of salt at 0° C, with the curves obtained at the same temperature in the presence of a constant concentration (0.1 M) of the anions of the acids.*

TABLE 3.—*Combination of wool protein with various acids, in the presence of a constant concentration of anions of the acids (0.1 M), at 0° C*

Hydrochloric acid[a]		Benzenesulfonic acid		Naphthalene-β-sulfonic acid	
pH	*Millimoles/g*	pH	*Millimoles/g*	pH	*Millimoles/g*
1.087	0.762	1.083	0.842	1.090	0.921
1.201	.789	1.391	.805	1.400	.912
1.380	.767	1.715	.814	1.721	.854
1.678	.745	2.038	.754	2.053	.810
1.988	.704	2.657	.693	2.378	.816
2.269	.664	3.515	.528	2.706	.787
2.535	.603	4.352	.334	3.451	.727
2.810	.538	5.276	.163	4.118	.627
3.206	.446	5.695	.090	4.400	.540
3.650	.357	6.45	.016	4.522	.495
4.210	.250	7.00	−.019	5.111	.392
4.617	.176	7.45	−.052	5.361	.303
5.032	.122			5.791	.209
5.473	.075			6.35	.083
5.867	.037				
6.15	.016				
6.3	.008				
6.4	.003				
6.5	.000				

[a] Data for hydrochloric acid at several other chloride concentrations have already been published.

the analysis of these data thus requires the development of a technique for the accurate estimation of this sum, the data obtained with Orange II at a constant concentration of its anion are not included in the present paper. The great steepness of the 0.1 *M* naphthalenesulfonate curve in figure 4 is due, in part, to combination with small amounts of salt at high pH values. These should have been added to the amounts of acid combined in order that the resulting curve should be described by eq 7.

4. EFFECT OF TEMPERATURE ON ANION AFFINITY

It has been shown [38] that a comparison of titration data obtained at two different temperatures permits an estimation of heat changes accompanying the dissociation of the protein-anion complex. Measurements at 25° C have therefore been made with four acids of widely different affinities. The results, summarized in table 4, also provide a basis of comparison for those acids which it was impractical to investigate at 0° C. Results obtained at 25° C with one of these acids, Orange II, are also included in the table. All of the data of table 4, combined with the data from table 1 for three of the acids at 0° C, are shown in figure 5. As the figure makes clear, the effect of temperature on the position with respect to pH of the curves obtained with picric acid (0.34 pH unit) is considerably greater than its effect on the curves for naphthalenesulfonic acid (0.21 unit), which in turn show a greater effect than the curves for hydrochloric acid (0.16 unit). No estimate is attempted of the effect of temperature on the position of curves for flavianic acid, since the data obtained at 0° C with this acid probably did not represent final equilibrium.*

TABLE 4.—*Combination of wool protein with various acids at 25° C, in the absence of added salt*

Hydrochloric acid		Naphthalene-β-sulfonic acid		Picric acid		Flavianic acid		Orange II [a]	
pH	*M-eq/g*	pH	*M-eq/g*	pH	*M-eq/g*	pH	*M-eq/g*	pH	*M-eq/g*
0.638	0.877	1.452	0.827	1.625	0.903	1.365	1.555	1.656	0.627
.826	.802	1.850	.757	1.776	.886	1.664	1.378	1.825	.624
1.448	.687	2.240	.734	2.055	.797	2.040	1.140	2.062	.627
1.710	.599	2.591	.608	2.359	.773	2.479	0.930	2.668	.592
1.915	.520	3.037	.401	2,402	.762	3.214	.710	4.308	.500
2.191	.402	3.415	.272	2.924	.622	3.830	.460	5.059	.334
2.406	.316	3.852	.167	3.414	.447	4.390	.328	5.934	.204
2.722	.225	4.422	.094	3.552	.431	4.775	.212	6.169	.133
3.093	.143			3.786	.304	5.090	.153	6.408	.0856
3.370	.087			4.094	.209	5.419	.075	6.421	.0530
3.962	.036			4.465	.140	5.800	.047	6.879	.0390
4.603	.014			4.868	.094	6.11	.022	6.926	.0280
5.257	.013			5.36	.054			6.72	.0175
6.375	.026			5.68	.033				
6.825	.035			6.604	.015				
7.275	.042								

[a] Equilibrium not attained. See text, p. 314.

*The curve at 25° C. for flavianic acid requires further explanation. When plotted, as in figure 5, as *milliequivalents* per gram against pH, the data run nearly parallel to those for picric acid at the same temperature up to values of acid combined of at least 0.65 millimole per g. This furnishes a very strong indication that in this range of pH values the flavianate ions combining with the wool are predominantly doubly ionized, and effectively neutralize two of the positively charged basic groups in the protein. At lower pH values where the data are represented by a broken line, the proportion of the total flavianic acid which is doubly dissociated is so small that a part of the ions which combine with the fiber are monovalent. In the most concentrated solutions, combination with the fiber takes place almost entirely with the monovalent ion, so that a plateau at 0.82 *millimole* rather than at 0.82 *milliequivalent* would presumably be found. Since the concentration of flavianic acid is extremely low in the range of pH in which all the ions are predominantly dibasic, it is clear that the affinity of the doubly charged anion is considerably higher than that of the monovalent ion. Trinitroresorcinol also combines with wool as a dibasic acid, when present in greater dilution. To avoid complicating figure 1, only the measurements representing the monobasic function of this acid were included in the figure. Similar effects appear to characterize sulfuric acid [34].

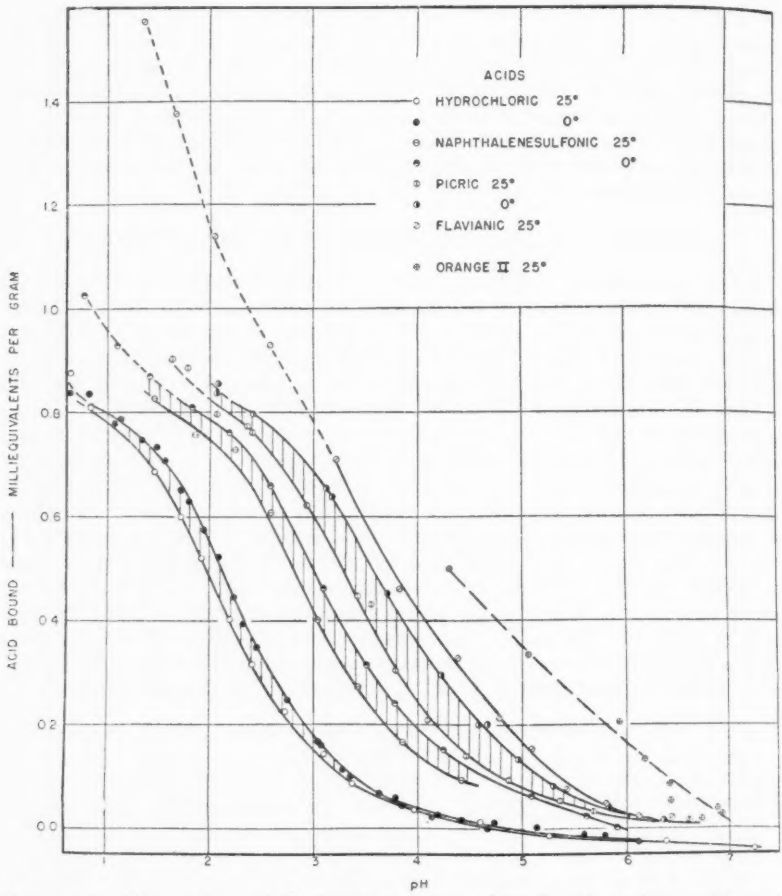

FIGURE 5.—*Comparison of the titration curves of wool with various acids at 0° and 25° C.*

TABLE 5.—*Calculation of anion affinity constants and heats of dissociation at 25° and 50° C*

Acid	pH of midpoint	$-\log K_A'$ from eq 8'	K_A'	Affinity relative to affinity of chloride	$\left(\frac{K_A'}{K_A'}\right)_0^{25}$	ΔH_0^{25} for anion dissociation
		25° C				*Calories*
Hydrochloric	2.16	0.31	0.49	----------	1.40	2,170
Naphthalene-β-sulfonic	3.03	1.97	.0107	46	1.81	3,850
Picric	3.52	2.89	.00129	380	2.80	6,670
Flavianic	4.07	[b] 3.77	.000170	2,880		
Orange II[a]	4.76	[c] 4.68	[c] .000021	[c] 23,400		
		50° C			$\left(\frac{K_A'}{K_A'}\right)_{25}^{50}$	ΔH_{25}^{50}
Hydrochloric	2.21	0.38	0.42	----------	0.85	−1,630
Orange II[a]	4.21	3.97	.000107	3,900	[c] 5.09	[c] 12,500

[a] Molecular weight, 328.3.
[b] Expressed for purposes of comparison as if the anion were monovalent. Refer to text.
[c] The Orange II data at 25° C do not represent equilibrium values. Thus the relative affinity for this temperature, and the value of ΔH_{25}^{50} are minimal.

The affinities at 25° C of the acids represented in figure 5, calculated from eq 8″, are given in the first part of table 5. The values of K_A' listed in column 4 were obtained graphically from the curve for 25° C in figure 2. Thus the effect of temperature on K_H' has been taken into account. Owing to the inclusion of data for Orange II, the differences of affinity comprised in this table cover almost eight times as wide a range as those listed in table 2.

The sixth column of the table gives the ratio of the protein-anion dissociation constants at 25° and 0° C. From this ratio, and the van't Hoff equation, the average heat of dissociation in this range of

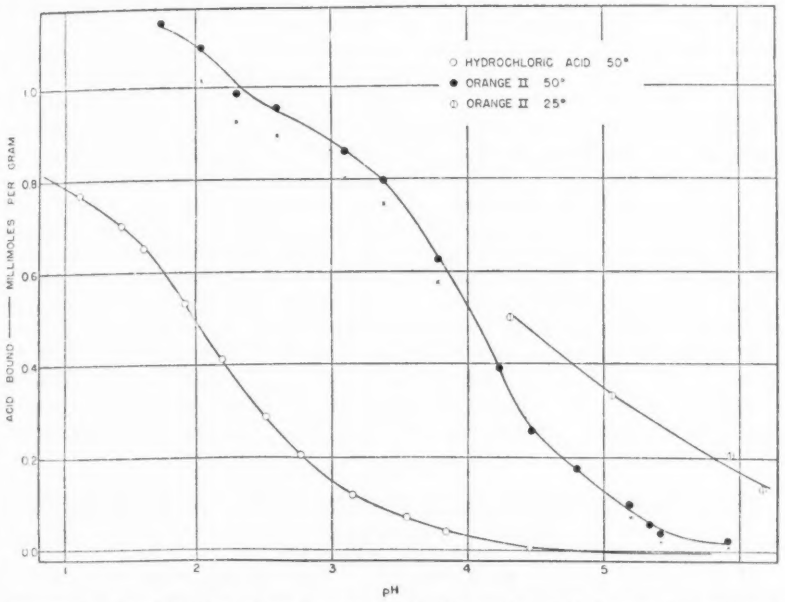

FIGURE 6.—*Combination of wool protein with the azodye acid Orange II, and with hydrochloric acid as a function of* pH *at 50° C.*

The significance of the crosses is explained in the text. For comparison, data obtained with Orange II at 25° C are included.

temperature has been calculated. The values so obtained are given in the last column. The heats of dissociation rise from some 2,000 calories with chloride ion to nearly 7,000 calories with picrate. As should be expected, more energy is required to dissociate the more tightly bound ions.[10]

In order to evaluate the effect of temperature on the affinity of Orange II, the amounts of this acid combined by wool were also measured at 50° C, after allowing a period of 5 days for the attainment of equilibrium. The results, together with data for hydrochloric acid at the same temperature are represented graphically in figure 6. The maximum amounts combined are in fair agreement with those reported for high concentrations of this dye by Ender and Müller [12]. Included in the figure for the purpose of comparison are the data

[10] The value of K_H' at 25° C was chosen on the basis of the earlier analysis of the effect of temperature on the combination of wool with hydrochloric acid [38], in which it was assumed that the heat of transfer of the ions from the solution to the fiber could be neglected. The heats of dissociation given in the table may therefore be subject to a small correction. Since this correction would be practically invariant, its application would not affect the relative order of the heats of dissociation, nor the great differences between them.

obtained with Orange II at 25° C. The measurements represented by crosses were obtained at 50° C by determining the increase in dry weight of the fibers as the result of combination with dye. The small discrepancies between the two sets of results (larger the more acid the solution) are accounted for by the fact that small amounts of amide nitrogen are split off, as a result of the prolonged exposure to dye at this temperature, and affect the apparent dye uptake estimated from the difference in the acid titer of the dye bath brought about by the wool. The amounts of ammonia produced by comparable exposure to the same concentrations of hydrochloric acid are smaller.

A comparison of the titration curve obtained with Orange II at 50° C with the portion of the curve obtained at 25° C strongly suggests that the data for the lower temperature do not represent a final equilibrium state. This was clearly the case when higher concentrations of dye were used at 25° C; combination of dye in 35 days at this temperature in no case exceeded 0.63 millimole, even when solutions more acid than those used at 50° C were employed. The affinity of the dye anion at 25° C (listed in the first part of table 5) must therefore be regarded as minimal. Thus, the heat of dissociation of the dye anions is even larger than the value in the table (12,500 calories), and exceeds by a large factor the heats of dissociation of the other ions listed. This large value also suggests an explanation of why the *rates* of dyeing and of stripping are so much affected by temperature. Since the anions are held much more loosely at elevated temperatures (the factor is greater than 5 in the interval 25° to 50° C), they are more readily passed on from one combining group in the fiber to another, toward the innermost parts of the fiber from the peripheral portions which are first dyed, or in the stripping process in the opposite direction.

IV. COMBINATION OF ANIONS WITH A DISSOLVED PROTEIN

If it can be shown that stoichiometric combination with anions occurs, in the reaction of both soluble and insoluble proteins with acids, there is a wide range of application in which it must be taken into account.[11] If it occurs only with wool or with fibrous proteins,[12] the distinction must be regarded as a clue to the nature of the specific structural differences between soluble and insoluble proteins.

To determine whether the marked differences in the titration curves of wool obtained with different acids are also to be found with dissolved proteins, determinations were made of titration curves of the soluble protein, egg albumin, with several of the same acids. The results of these measurements at 22° C are summarized in table 6 and shown in figure 7. The position of the broken line at the top of this figure, inserted for reference, shows the maximum acid-binding capacity, indicated by the work of Kekwick and Cannan with hydrochloric acid [22]. The crosses distinguish measurements in which incipient precipitation was indicated by a faint opalescence. In every case measurements with higher concentrations of the acid produced a visible precipitate, and have been omitted from both the

[11] Examples are (a) the calculation of Donnan membrane potentials or ion-distribution ratios, in which disagreement with experiment often appears [3, 4] unless combination with ions other than H^+ and OH^- is taken into account [1]; (b) the possibility of forming physiologically significant weakly dissociated protein complexes, such as with calcium [10].

[12] The amounts of acid or base combined with *silk* at any pH [13] have the same first-order dependence on the concentration of anions and cations, respectively, that characterizes wool.

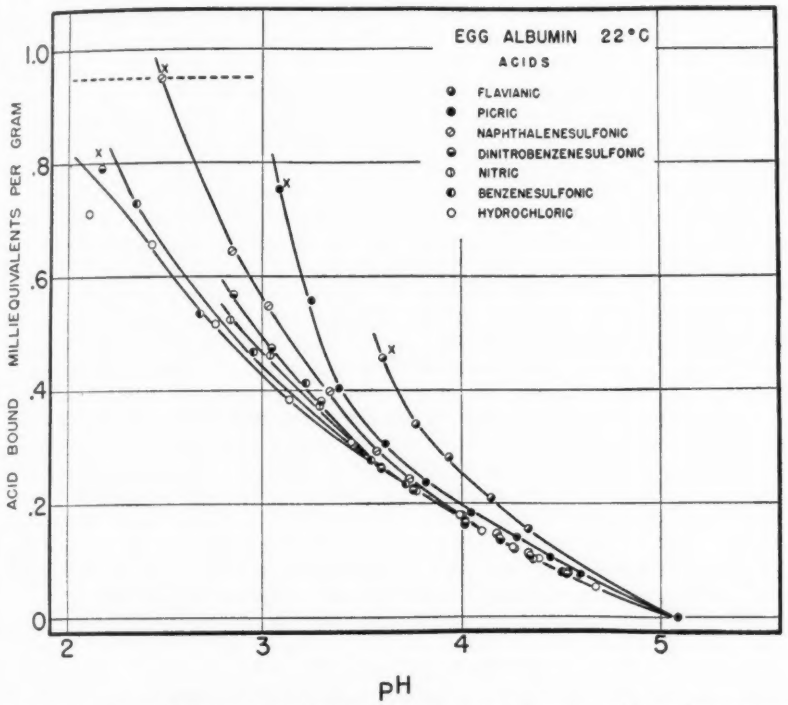

FIGURE 7.—*Combination of a dissolved protein, egg albumin, with various strong acids as a function of* pH.

The significance of the "crosses" is explained in the text.

TABLE 6.—*Combination of a dissolved protein, egg albumin, with various acids at 22° C, in the absence of added salt*

Hydrochloric acid		Benzenesulfonic acid		Nitric acid		2,4-Dinitrobenzenesulfonic acid		Naphthalenesulfonic acid		Picric acid		Flavianic acid	
pH	*M-eq/g*	pH	*M-eq/g*	pH	*M-eq/g*	pH	*M-eq/g*	pH	*M-eq/g*	pH	*M-eq/g*	pH	*M-eq/g*
2.109	0.710	2.362	0.728	2.840	0.525	[a] 2.181	0.790	[a] 2.488	0.942	[a] 3.086	0.762	[a] 3.608	0.456
2.438	.658	2.688	.523	3.041	.462	2.499	.621	2.848	.645	3.248	.564	3.772	.340
2.766	.524	2.959	.468	3.282	.371	2.859	.568	3.028	.550	3.385	.404	3.939	.282
3.140	.392	3.220	.411	3.598	.262	3.049	.474	3.340	.396	3.618	.303	4.150	.211
3.548	.277	3.450	.309	3.764	.221	3.298	.380	3.580	.292	3.825	.237	4.338	.155
3.777	.222	3.726	.236	4.028	.163	3.599	.262	3.740	.244	4.047	.185	5.092	.000
3.994	.181	4.020	.169	4.198	.135	3.760	.224	4.009	.180	4.282	.141		
4.105	.152	4.273	.120	4.360	.102	4.022	.166	4.181	.148	4.450	.105		
4.260	.123	4.515	.079	4.559	.074	4.196	.139	4.339	.116	4.600	.076		
4.393	.103	5.092	.000	5.092	.000	4.348	.109	4.537	.081	5.092	.000		
4.677	.055					4.535	.076	5.092	.000				
5.092	.000					5.092	.000						

[a] Solution opalescent. Higher concentrations of acid produced a visible precipitate. Measurements made at these higher concentrations are therefore omitted from the table.

table and the figure in order to avoid all possibility that the effects described may have been due, in whole or in part, to the existence of two macroscopic phases.

The differences between the curves shown in figure 7 obviously correspond to the differences found with wool. With one exception, the affinities of the anions for egg albumin are in the same order as

their affinities for wool. One difference, however, distinguishes the two sets of data. The positions of points along the curves for egg albumin show no tendency to remain equidistant with respect to the pH axis; neighboring curves converge strongly as decreasing amounts of acid are bound. This convergence may be regarded as a consequence of the excess of carboxyl groups over amino groups in the dissolved protein. Because of this inequality only a part of the carboxyl groups are ionized in the uncombined state, and this part alone is free to accept hydrogen ions from the acids. The complete titration curve of all the carboxyl groups of the protein extends beyond the isoionic point and can only be obtained by titration with base as well as with acid. The part of the carboxyl curve which is obtained by combination with base in the absence of salt is not governed by the affinity properties of anions, since the cations of bases rather than the anions of acids are present when it is obtained. Thus, unless salt is present, the influence of the anion disappears entirely at the point of zero combination with acid. This circumstance tends to obscure the simplicity of the theoretical relation between the affinity of the anion and the pH coordinates of acid titration curves. This simplicity will manifest itself only when the acid combination curve coincides with the titration curve of the carboxyl groups as a whole, as it does with wool. With salt present a secondary effect of the anion beyond the point of zero combination may be observed, as the data of Briggs [5] obtained on serum albumin with metaphosphate plainly show.

Since the curves in figure 7 converge sharply, it is difficult to compare the absolute magnitudes of the differences in affinity of the several anions with the corresponding differences found with wool. Obviously, the comparison should be made between solutions which are as far as possible from the isoionic point in order to minimize the effects of the convergence. The data do not extend to very acid solutions, but at the extreme of the range represented the differences in the pH coordinates of the curves approach values only slightly smaller than the differences between curves for the same acids in figure 1.[13]

Since the theory of stoichiometric anion combination was originally formulated to account for the large effects of the presence of salt on the hydrochloric acid combination curves of wool, the application of this theory to the much smaller salt effects described by Cannan for egg albumin [6] seemed at first uncalled for. The experiments just described, however, suggest that the difference between the titration curves of dissolved and undissolved proteins in the absence of salt [36] cannot be due to the failure of anions to combine with the dissolved protein. The contradiction between the results of the two kinds of experiments is only apparent, since the existence of a stoichiometric salt effect is not an inevitable consequence of every situation to which eq 1 may be applied. Such salt effects are found only when $K_A' \gg a_A \gg K_A$. When K_A' and K_A are both very large, the bracketed term in the denominator of eq 1 approaches a constant and is nearly independent of a_A. When both constants are very small, the bracketed term approaches unity and is again nearly independent of a_A. In

[13] Results of the kind described for both proteins may furnish a basis for explanations of the many reports of ion specificity in the literature of protein and enzyme chemistry. Attempts have been made to deal with some of these specific properties, such as differences in salting-out effectiveness, by treatment of the electrostatic interaction between ions having different sizes, shapes, and electric moments. Many phenomena of specificity remain, however, for which plausible explanations, based on simple electrostatic considerations alone, are not easily obtained. Among these are numerous instances of differences in the electrophoretic mobility of various proteins when different anions or different concentrations of anions are present in buffers [9, 14, 31, 39], and large-scale differences in the effectiveness of various anions in environments producing protein denaturation [15, 35].

either case (corresponding to extremely low or extremely high affinity of anions for the protein) the availability of hydrogen ions rather than of anions becomes the factor limiting the extent of combination with acid. Thus, as in the case of egg albumin, the titration curve obtained in the absence of salt will not differ greatly from those obtained at constant anion concentrations.

In view of the numerous indications that dissolved proteins do combine with such commonly employed anions as chloride, acetate, and phosphate, but not to an extent comparable to their combination with hydrogen ions in acid solutions [1, 3, 14, 39], the first of the two alternatives (K_A' and K_A large) probably applies in the case of these simpler ions. The difference in the absolute values of these constants characterizing the soluble and insoluble proteins may be related to the structural factors responsible for the differences in their respective states of dispersion.

It should be noted in figure 7 that precipitation occurred at concentrations which were in the inverse order of the affinities. Picrate and flavianate are ions having a high precipitating efficiency for proteins, but many others, such as tannate and metaphosphate, are equally well known [28, 29, 26, 5]. It would be of interest to determine to what extent protein precipitation by specific reagents, such as picrate or tannate, and the salting out of proteins with various salts may be regarded as manifestations of the same general protein-anion equilibrium. When the affinity is so great that the presence of only small amounts suffices to cause combination and precipitation, the anion or acid is naturally described as a specific protein precipitant. When the affinity is low and large excesses are required, the combination and precipitation may have many of the characteristics associated with salting out. With extremely low affinities, such as that of chloride, salting out is often not practicable.

V. THE RELATION OF ANION AFFINITY TO SIZE, STRUCTURE, AND BASICITY OF IONS

If the phenomena reported in the present paper are to be ascribed to the formation of partially dissociated protein-anion combinations, it is logical to inquire into the nature of the forces which confer on different ions so wide a range of affinities for protein. The existence in certain kinds of solutions of ion combinations which are only partially dissociated is now well established [7, 24]. Such combinations may be formed in two possible ways, involving either directed or undirected valence forces. The first alternative involves the formation of a bond having a partially covalent nature, or a hydrogen bond; the second comprises various kinds of polar forces, whether electrostatic (coulombic) in nature or otherwise and includes van der Waals forces. If the first type is involved, the principal relation found among the anions should be an inverse one of affinity to the intrinsic strength of the acid.[14] The relations found in cases to which the second alternative applies would be expected to be more complex.

Since most of the acids included in the present research are of approximately equal strengths in water, but differ widely in affinity, the first alternative seems to have little in its favor. Nevertheless, a *part* of the total binding energy between anion and protein might

[14] Here the possibility of the formation of a bond between the protein and the charged group of the anion (when the anion is polyatomic) is alone under consideration.

still be due to the formation of a directed bond, especially since the intrinsic strengths of the various acids, as distinguished from their apparent strengths in water, are not all equal.

In an effort to appraise more closely the influence of the basicity of the anion on the affinity, comparisons (fig. 8) have been made of the results obtained with pairs of closely similar acids, such as benzenesulfonic and benzoic acids, which differ widely in strength. The data shown are limited to ranges of pH in which the concentration of the undissociated form of the weak acid does not exceed 0.1 *M*, in order to avoid effects, due to the presence of larger amounts, which have been described elsewhere [37]. There is very little difference

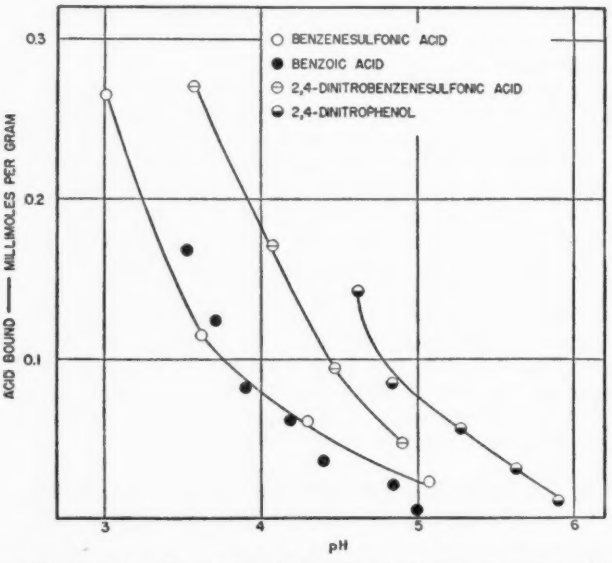

FIGURE 8.—*Comparison of portions of the acid titration curves of wool protein obtained with pairs of analogous acids which differ widely in strength.*

between the amounts of strong and of weak acid combined at a given pH in the case of the benzenesulfonic-benzoic acid comparison. On the other hand, the anion of dinitrophenol appears to have a definitely higher affinity for wool than the anion of its totally dissociated analogue, dinitrobenzenesulfonic acid. The opposite situation is found in experiments (still in progress) on a series of aliphatic carboxylic acids. These have even lower affinities for wool than has hydrochloric acid; in addition, the weaker acids have lower affinities than the relatively strong members of the series. The affinities of all of them are increased by the introduction of a halogen or of a hydroxyl group, although the derivatives thus formed are considerably stronger acids. Since there is no consistency in these results, it seems likely that the differences found are due to specific structural effects rather than to differences in acidity.

An indication of the nature of the *undirected* forces to which the principal recourse must therefore be had is to be found in the fairly regular correspondence between the order of affinities and of molecular

weights, shown in table 2. Such a dependence on the mass of the anion is consistent with the Fajans rule, which deals with the degree of ionic character of bonds and their consequent tendency to dissociate. However, a dependence on mass, or functions of ion size related to it, is also generally characteristic of similar phenomena which depend, at least in part, on van der Waals forces, or adsorption [19].

The several exceptions to a strict correspondence between the relative masses of the ions and their affinities provide further information as to the relation between structure and affinity. Thus the affinities of the aliphatic carboxylate ions already mentioned do not fall into a molecular weight order; likewise, ethylsulfate ion has as low an affinity as the much smaller chloride ion, and is lower in relative affinity than the smaller bromide and nitrate ions. The anion of trinitroresorcinol is slightly lower in affinity than picrate, although it is slightly heavier. Napthalenesulfonate is higher in affinity than the heavier dinitrobenzenesulfonate ion.

A possible way of resolving these discrepancies without introducing a host of special assumptions is suggested if due regard is had for the shapes of the various ions and the character of the surfaces which they present to the protein. Two-dimensional ions (those derived from simple benzenoid compounds) appear to possess higher affinities than either compact three-dimensional or chain-like particles of the same mass. The nature of the substituents added appears to be of less importance than their mass as long as there is no steric hindrance to their lying in the plane of the ring. Such a hindrance appears to operate in the case of the monovalent trinitroresorcinol ion, which has an appreciably lower affinity than picrate. It appears doubtful, from the study of models, whether the undissociated hydroxyl group in this ion can lie in the plane of the ring. When the flat configuration is imposed by the existence of conjugated double bonds, as in naphthalenesulfonic acid, the affinity relative to a disubstituted benzenoid compound of the same mass gains accordingly. Wide differences between the degree of substantivity of *o*- and *m*-substituted benzidine azo dyes cited by Robinson and Mills [27] furnish extreme examples which are entirely consistent with the present suggestion.

The fact that ion flatness appears to favor affinity for protein need not be interpreted as an exclusive dependence of affinity on the size of the surface presented by an ion. Saturated aliphatic hydrocarbon chains in an ion probably do not exercise any considerable attractive forces on the fragments of hydrocarbon chains which exist on the surface of the protein. A close approach between them is obstructed by the outer shell of hydrogen atoms common to both. Thus, the greater affinity of ions composed of aromatic hydrocarbons may be due to the absence of this obstructive shell on two of their largest surfaces. If, in addition, electronegative substituents, such as chlorine, or the oxygen of nitro groups, are presented by the ion to the protein, strong attractive forces may arise, replacing the relative indifference which determines the behavior toward protein of the unsubstituted hydrocarbon. Experiments designed to test these views, and the possibility of applying them in the deliberate control of affinity, are at present in progress.

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EFFECT OF THE QUENCHING RATE ON SUSCEPTIBILITY TO INTERCRYSTALLINE CORROSION OF HEAT-TREATED 24S ALUMINUM ALLOY SHEET

By Hugh L. Logan

ABSTRACT

The effect of the quenching rate of the 24S aluminum alloy upon its behavior in an accelerated corroding solution ($NaCl+H_2O_2+H_2O$) was determined. Specimens cooled from a temperature above 400° C at a rate in excess of 815° to 1,110° C/sec (increasing with increasing copper content of the alloy) were susceptible only to the pitting type of corrosion. Specimens cooled at a rate less than 300° to 400° C/sec (increasing with increasing copper content) and specimens cooled slowly to a temperature below 400° C before quenching were subject to severe intercrystalline corrosion. For cooling rates between the above ranges there was a gradual change from one type of corrosion to the other.

Cooling rates were determined by means of a thermocouple and an Einthoven string galvanometer.

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I. INTRODUCTION

There are two commercial aluminum alloys of the duralumin type that are widely used in aircraft structures in this country. Both alloys are ordinarily used in the heat-treated condition. The principal alloying constituents are copper, magnesium, manganese, and silicon, the chief difference in composition of the two being in the copper and magnesium content. Because of its higher strength, the alloy containing the relatively larger amounts of copper and magnesium is now usually preferred in aircraft structures. The designation of this alloy, 24S, by its manufacturer, the Aluminum Company of America, is generally used in referring to this material.

The recommended heat treatment for any aluminum alloy consists in: (a) heating to form a solid solution of the essential alloy constituents in the aluminum; (b) cooling rapidly from the solution tempera-

ture to approximately room temperature; (c) aging at room temperature or at some optimum elevated temperature. The recommended solution heat-treatment temperature for the 24S alloy is 493° ± 5° C. The aging effect (hardening) is completed in about 4 days at room temperature.

Without surface protection, this alloy is not immune to corrosion, and the severity of the corrosion damage varies greatly according to the environment. The most damaging corrosion is that which penetrates along the grain boundaries, causing a significant decrease in strength and ductility out of all proportion to the outward, visible evidence of the severity of the damage. However, if cooled from the solution temperature at a sufficiently rapid rate, the alloy is immune to intercrystalline corrosive attack, even in severely corrosive environments.

Very little published information is available concerning the rates, in quantitative terms (degrees per unit of time), at which it is necessary to cool the 24S alloy from the solution temperature in order to avoid susceptibility to intercrystalline corrosion. In the recommended procedures for the heat treatment, the most specific statement concerning the cooling rate is that the alloy shall be "quenched in cold water." In industrial heat-treating operations, in which large quantities of material are handled at one time, wide variations in cooling rates are to be expected—not only because of differences in the temperature of the cooling water but also because of unavoidable difficulties in cooling every portion of a large piece, or every piece of a large batch, uniformly at the same rate, even if the cooling water is at a properly "cold" temperature. Procedures other than plunging the material into cold water may cool the alloy at rates entirely too slow to provide immunity to intercrystalline corrosion.

Drastic cooling, in the endeavor to render the alloy immune to intercrystalline corrosion, may result in distortion of the quenched material and necessitate expensive straightening operations. In many cases it would be desirable to fabricate a structure from material in the soft or annealed condition and subsequently to heat-treat it, if the structure could be cooled slowly enough to minimize distortion and still rapidly enough to insure immunity to intercrystalline corrosion.

The strength and hardness of the heat-treated alloy are not significantly affected by the cooling rate for a wide range of rates, including those which render the alloy susceptible to severe intercrystalline corrosion. Results of tensile and hardness tests generally do not indicate whether or not the alloy was cooled from the solution temperature with sufficient rapidity to prevent intercrystalline corrosion.

This report [1] describes the method used at the National Bureau of Standards for determining cooling rates of specimens of 24S aluminum alloy sheet cooled from the recommended solution temperature by "quenching" in water maintained at different temperatures, and also gives results of a study to determine the relations between the cooling rates and susceptibility to intercrystalline corrosion. The minimum cooling rates that would provide immunity to intercrystalline corrosion were also determined for the specimens available.

[1] A preliminary report was presented before the American Physical Society, April 29, 1939. Phys. Rev. 55, 1139 (1939).

II. EXPERIMENTAL PROCEDURE

1. MATERIALS

It is generally believed that the mode of combination and distribution of the copper, rather than of the magnesium and manganese, in the alloys of the duralumin type, is the basic factor that determines whether or not the alloys are susceptible to intercrystalline corrosion, and furthermore, that the rate of cooling from the solution temperature has an important effect on the mode of combination and distribution of the copper in these alloys. It was considered important in determining the critical cooling rates of the commercial 24S alloy to obtain data on alloys of different copper contents. Through the courtesy of the Aluminum Company of America, sheet material, 0.040 in. thick, and in the annealed condition, was furnished in the compositions given in table 1.

TABLE 1.—*Compositions of 24S alloy sheet*

Alloy number	Copper	Magnesium	Manganese	Iron	Silicon	Aluminum (by difference)
	%	%	%	%	%	%
A05	4.05	1.49	0.64	0.28	0.19	93.35
A27	4.27	1.51	.65	.27	.19	93.11
A64	4.64	1.61	.63	.27	.21	92.64

2. DETERMINATIONS OF COOLING RATES

Specimens ½ by ½ in. were cut from the 0.04-in. sheets of the three compositions given in table 1. The temperatures of the specimens during heat treatment were measured with Chromel-Alumel thermocouples peened into 0.018-in. holes drilled into the specimens, as shown in figure 1. The thermocouple circuit was completed through the specimen itself, and the results are based on the assumption that the indicated temperature is that at the surface of the specimen, at the point of contact. Calculation shows that the surface temperature in a specimen of the dimensions used does not differ by more than 3° C from that of the center, for the cooling rates plotted in figure 3. The specimens were heated in air in a vertical electrical resistance tube furnace at the solution temperature (493° ±5° C) for 30 minutes. The thermocouple wires and insulating tubing served as a support for lowering the specimens into the quenching bath placed immediately below the furnace tube.

The rapid changes in temperature that occurred in the specimens during quenching were recorded photographically as time-temperature cooling curves by means of an Einthoven string galvanometer and auxiliary apparatus shown in figure 2. The thermocouple wires from the specimen were connected in series with the galvanometer string, which was a tungsten wire (0.0002- to 0.0003-in. diameter) located vertically in the horizontal magnetic field in the galvanometer, *G*. The current in the thermocouple circuit caused deflections of the "string" in the magnetic field that (resistance of the circuit remaining constant) are proportional to the emf, and therefore to the temperature, inasmuch as the temperature-emf relation of the thermocouple

is practically a straight line. The computed change in the resistance of the thermocouple, in the range 20° to 500° C, was so small compared to the resistance of the whole circuit that it was within the experimental error and could be neglected. The motion of the "string", magnified 500 times, was recorded by its shadow in the beam of light coming through the lens system *C–C'*, on a moving strip of photographic paper in the camera, *R*. A rotating sector, *I*, driven in synchronism with an electric tuning fork, interrupted the light beam at intervals of 0.04 second and thus produced a time scale on the recording paper. A grating mounted in the camera reproduced the temperature scale. Time intervals could be estimated to 0.01 second and temperature to 2½° C. The apparatus was calibrated before the quenching of each specimen by recording on the photographic paper the zero position of the string (zero emf) and the position of the string when the emf (determined by a potentiometer) of the thermocouple at the heat-treating temperature was applied to it. The use of this apparatus for measuring cooling rates of steels in different quenching media has been described elsewhere.[2] [3]

To obtain different quenching rates on specimens of the same size, quenched from the same temperature, it was necessary to change the temperature of the quenching bath. Obviously the lower the temperature of the quenching bath, the more rapid the cooling rate for specimens of the same size. In water at 22° C, quenching rates as high as 10,000° C/sec were obtained; and in water at 80° C, the cooling rates were reduced to approximately 1,100° C/sec.

The cooling rates obtained in this investigation at the various quenching water temperatures would not necessarily be obtained with specimens of different sizes or shapes, or with a mass of water which was not large compared with the mass of the specimens.

Typical cooling curves obtained by quenching specimens in water at different temperatures are shown in figures 5 and 6. The temperature of the specimens decreased very slightly (3° to 10° C) between the time of withdrawal from the furnace and the time of quenching. The specimens cooled rapidly during the quenching until, after 0.05 to 3 seconds, they had reached a temperature of approximately 120° C. There was then an abrupt decrease in the cooling rate, and during the next 3 to 10 seconds the specimens reached the temperature of the quenching medium.

The cooling rates given in this paper are average rates for the interval between the beginning of the "quench," approximately 490° C, and the temperature at which rapid cooling ceased, 115° to 125° C in most cases.

Numerous experiments indicated that the rate of cooling below a temperature of about 120° C had little or no influence on the susceptibility of the 24S alloy sheet to intercrystalline corrosion, provided the cooling rate above this temperature was sufficiently rapid.

All of the specimens used in the work described here were quenched in a quantity of water large enough with respect to the mass of the specimen so that no significant temperature increase occurred in the water. With the small specimens used (½ by ½ by 0.04 in.) it can be shown by calculation that the temperature gradients in the specimens

[2] H. J. French and O. Z. Klopsch, *Quenching diagrams for carbon steels in relation to some quenching media for heat treatment.* Trans. Am. Soc. Steel Treating **6**, 251 (1924).
[3] T. G. Digges, *Influence of austenitic grain size on the critical cooling rate of high-purity iron-carbon alloys.* J. Research NBS **24**, 723 (1940) RP1308.

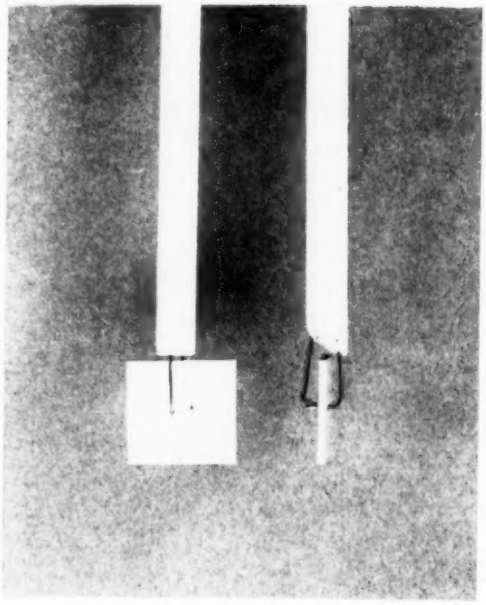

FIGURE 1.—*Arrangement of thermocouple wires on specimen during heating and cooling cycle.*

Wires serve as support for specimen in furnace and in quenching bath. ×1.

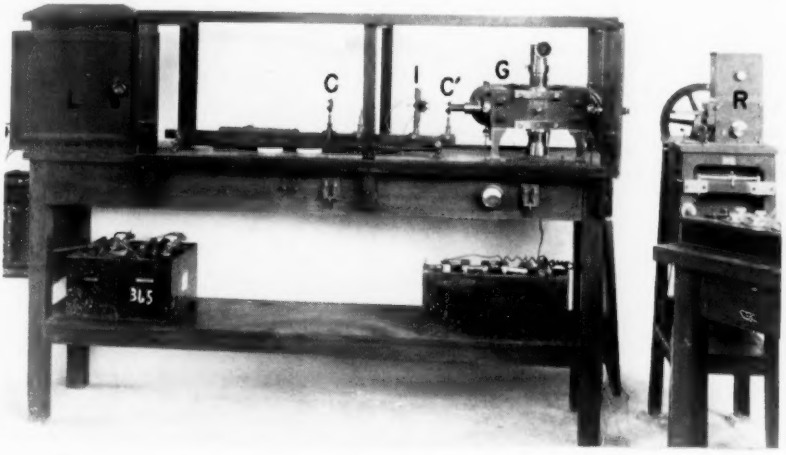

FIGURE 2.—*Einthoven string galvanometer and photographic recorder.*

G, galvanometer with "string" mounted in the large vertical tube. *C*–*C'*, lens system for light beam from *L*. *I*, sector disk interrupting the light beam to establish time intervals. *R*, camera containing moving strip of photographic paper.

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were not great enough to make any significant difference between the cooling rate of the interior of the specimen and that at the sides, where the thermocouple wires were attached.

3. CORROSION TESTS

To determine the differences in corrosive attack on the alloy cooled at different rates, the specimens, after aging at room temperature, were immersed for 48 hours in a water solution containing 57 g of NaCl and 100 ml of H_2O_2 (3-percent solution) per liter. The solution was renewed after the first 24-hour period. The kind and extent of the corrosion damage were observed by metallographic examination of cross sections of the corroded specimens. Although the severity of the corrosion obtained in a laboratory test of this kind cannot be correlated with that obtained in service for any given length of time, the test is considered reliable to show whether or not the material is susceptible to intercrystalline corrosion in a severely corrosive environment.

III. RESULTS

1. EFFECT OF COOLING RATE UPON SUSCEPTIBILITY TO INTERCRYSTALLINE CORROSION

The unprotected 24S alloy in any condition was severely corroded during 48 hours' immersion in the oxidizing salt solution. The corrosion obtained on the various specimens was divided into three general classes, namely: (1) pitting; (2) pitting and traces of intercrystalline corrosion; (3) severe intercrystalline corrosion. The type of corrosion obtained with any particular specimen depended primarily upon the cooling rate and the copper content of the alloy. The results obtained on the specimens investigated are given in table 2 and figure 3.

TABLE 2.—*Cooling rates obtained on specimens of 24S aluminum alloy sheet and corresponding types of corrosion*

Copper content	Range of cooling rates	Quenching water temperature	Number of specimens	Number of specimens susceptible to—		
				Pitting corrosion only	Pitting and traces of intercrystalline corrosion	Severe intercrystalline corrosion
%	°C/sec	°C				
4.05	815 to 1,325	83 to 86	12	12	0	0
4.27	935 to 10,000	22 to 87	13	13	0	0
4.64	1,110 to 1,645	80 to 83	4	4	0	0
4.05	540 to 785	85 to 87	8	5	3	0
4.27	585 to 875	84 to 90	13	6	7	0
4.64	690 to 1,090	83 to 86	11	7	4	0
4.05	305 to 525	84 to 93	12	0	10	2
4.27	305 to 545	85 to 91	10	0	8	2
4.64	405 to 670	84 to 91	11	0	8	3
4.05	145 to 260	93 to 95	4	0	0	4
4.27	95 to 300	90 to 98	12	0	0	12
4.64	120 to 395	85 to 99	4	0	0	4

On specimens quenched at rates greater than those represented by curve *A*, figure 3 (815° to 1,110° C/sec), only the pitting type of attack

was obtained with no intercrystalline penetration from the bottoms of the pits. This type of corrosive attack is illustrated in figure 4, *A* and *B*, showing cross sections of corroded sheet.

Severe intercrystalline corrosion was obtained on the specimens cooled at rates falling on or below curve *C*, figure 3. Typical examples are shown in figure 4, *E* and *F*. The corrosive attack was very severe at the grain boundaries and resulted in a general loss of cohesion, with entire grains or crystals dropping away from the main body of the

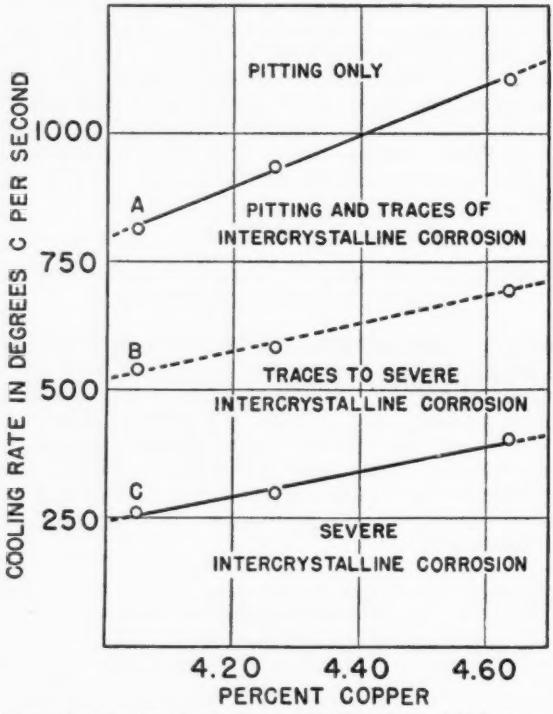

FIGURE 3.—*Type of corrosion obtained on 24S alloy sheet of different copper contents quenched at different rates.*

Specimens cooled at rates above curve *A* were immune to intercrystalline corrosion; rates between curves *A* and *B* made specimens susceptible to pitting or to pitting with traces of intercrystalline corrosion; specimens cooled at rates below curve *B* were susceptible to intercrystalline corrosion in some degree, and at rates below curve *C*, to severe intercrystalline attack.

material. The cooling rates on or below curve *C* (260° to 395° C/sec) all of which produced the condition resulting in severe intercrystalline attack, were obtained by quenching the specimens in water near the boiling point. Cooling rates obtained by allowing the specimens to cool from the solution temperature in still air, or in the air blast from a fan (velocity 900 to 1,200 ft/min), were 5° to 12° C/sec. These specimens also were susceptible to very severe intercrystalline corrosion.

There was a gradual change from pitting to the predominantly intercrystalline type of corrosion in the specimens of the three compositions of table 1, as the cooling rates were decreased from the values on or above curve *A* to those on or below curve *C*, figure 3.

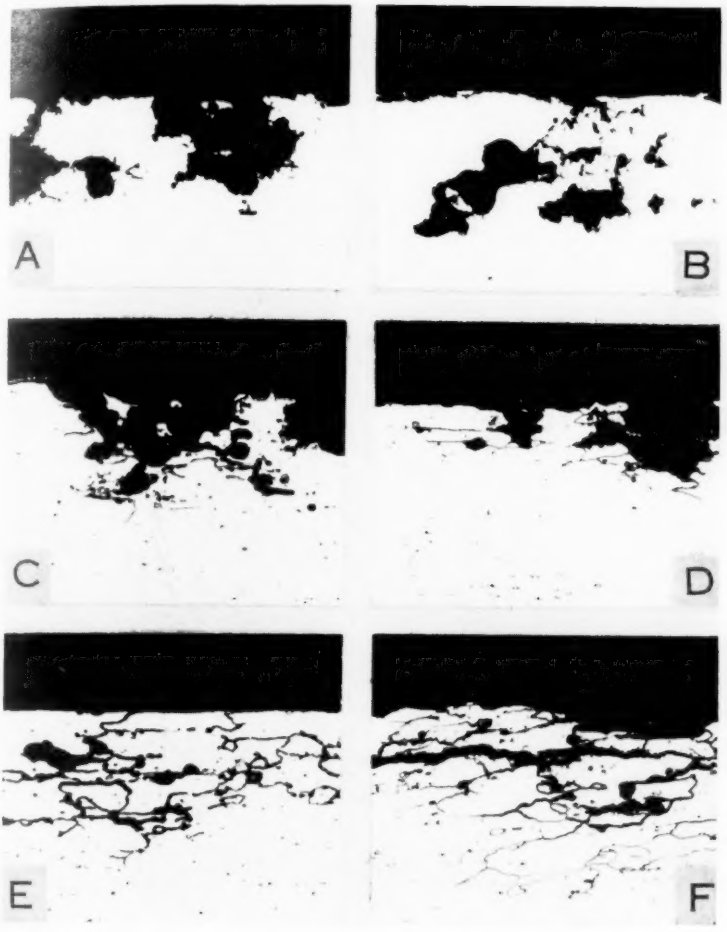

FIGURE 4.—*Cross sections of specimens of 24S aluminum alloy sheet showing penetration of corrosion from the exposed surfaces of the sheet, after immersion in the* $NaCl+H_2O_2$ *solution.*

A and *B*, pitting attack; *C* and *D*, traces of intercrystalline corrosion at the bottoms of pits; *E* and *F*, severe intercrystalline corrosion. Unetched. ×100.

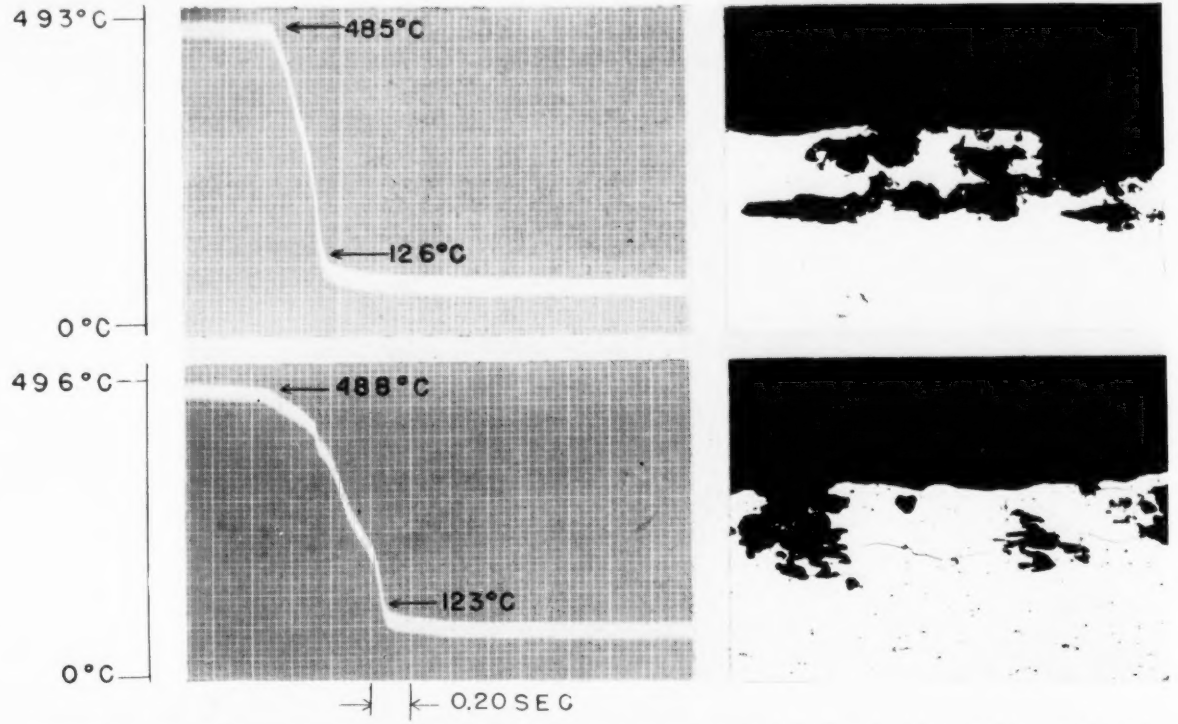

FIGURE 5.—*Typical cooling curves and the kind of corrosion obtained on the 24S alloy sheet in oxidizing salt solution.*

Upper curve, cooling rate 1,330° C/sec, between 485° and 126° C; temperature of quenching bath, 80° C; pitting corrosion. Lower curve, cooling rate 900° C/sec, between 488° and 123° C; temperature of quenching bath, 90° C; pitting with traces of intercrystalline attack. Photomicrographs show sections normal to corroded surface. Unetched. ×85.

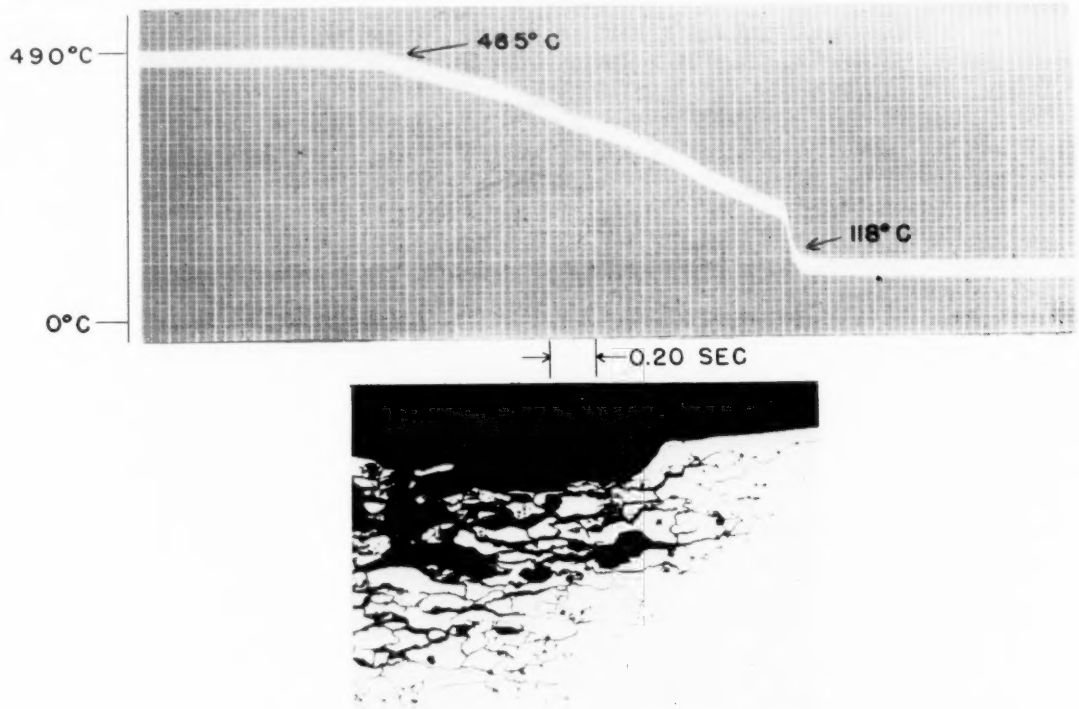

FIGURE 6.—*Typical cooling curves and the kind of corrosion obtained on the 24S alloy sheet in oxidizing salt solution.*

Cooling rate 205° C/sec, between 485° C and 118° C. Temperature of quenching bath, 97° C. Severe intercrystalline corrosion. Photomicrograph shows section normal to corroded surface. Unetched. ×85.

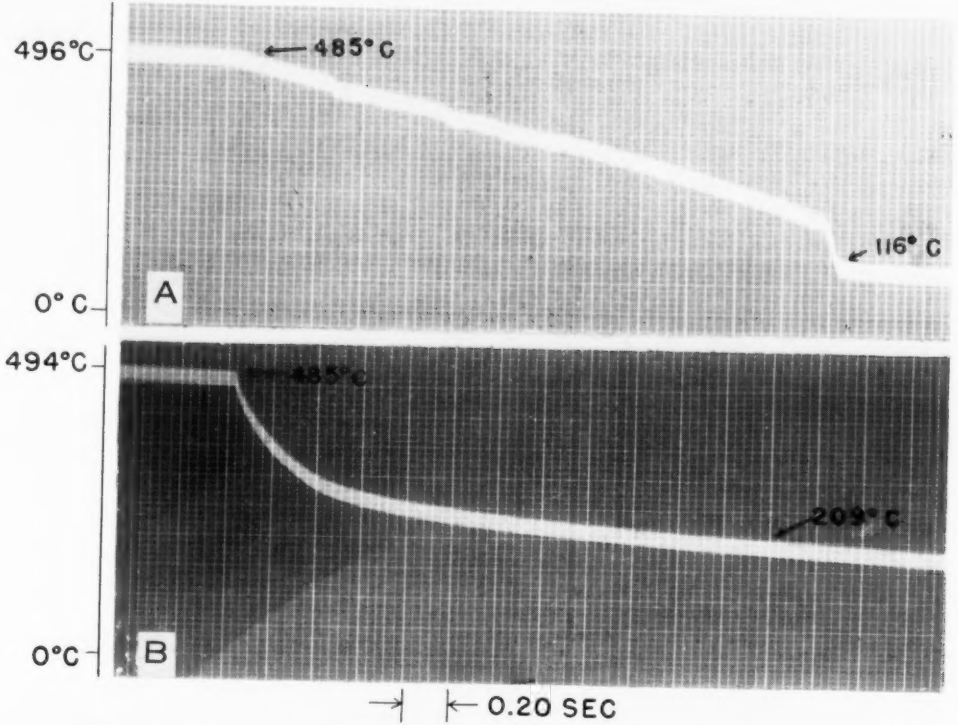

FIGURE 7.—*Cooling curves obtained by quenching in water and in oil, at 98° C.*

Lack of inflections in oil-quench curve *B* indicate that inflections in water-quench curve *A* were caused by formation and rupturing of steam layer on surface of specimen in water.

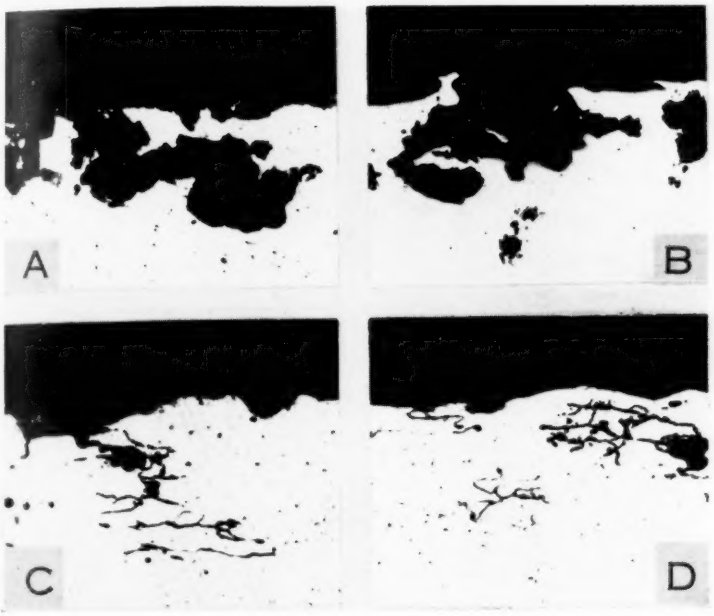

FIGURE 8.—*Type of corrosion of 24S alloy quenched in water after an intermediate air cooling following withdrawal from the furnace.*

Photomicrographs show sections normal to corroded surfaces. Unetched. ×100.

A, quenched from 405° C in water at 44° C; cooling rate 5,950° C/sec; pitting only. *B*, quenched from 400° C in water at 21° C; cooling rate 4,700° C/sec; pitting only. *C*, quenched from 398° C in water at 25° C; cooling rate 9,500° C/sec; pitting with traces of intercrystalline corrosion. *D*, quenched from 394° C in water at 43° C; cooling rate 5,500° C/sec; severe intercrystalline corrosion.

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For the cooling rates lying between curves *A* and *B*, the corrosion was predominantly pitting, with occasional specimens having traces of intercrystalline corrosion at the bottoms of the pits, as illustrated in figure 4, *C* and *D*. For the cooling rates lying between curves *B* and *C*, figure 3, all of the specimens contained some intercrystalline corrosion, the attack varying from traces at the bottoms of pits, as shown by *C* and *D*, figure 4, to the generally severe intercrystalline attack, such as is shown in *E* and *F*, figure 4.

It should be pointed out that there is no sharp line of demarcation between cooling rates that give one type of corrosion and another. For example, on certain specimens there were no traces of intercrystalline corrosion for cooling rates as low as curve *B*, figure 3, and on others there were traces of intercrystalline corrosion for cooling rates as high as those lying on curve *A*. The results obtained indicate that the minimum cooling rates which render the materials immune to intercrystalline corrosion lie along curve *A*.

Shown with the cooling curves, figures 5 and 6, are examples of the kinds of corrosion found on the heat-treated specimens after immersion in the oxidizing salt solution. The upper cooling curve in figure 5 was obtained on a specimen of the 24S alloy containing 4.27 percent of copper, quenched at a rate of 1,330° C/sec. This rate is well above curve *A*, figure 3, and the specimen immersed for 48 hours in the oxidizing salt solution was pitted, with no traces of intercrystalline corrosion.

The lower cooling curve in figure 5 was obtained on a similar specimen (4.27 percent of copper) quenched at a rate of 600° C/sec, which is just above curve *B*, figure 3. The corroded specimen, as shown by the photomicrograph, was pitted, with slight intercrystalline attack extending from the bottoms of the pits.

The curve shown in figure 6, was obtained on a specimen quenched at an average rate of 205° C/sec, a rate well below curve *C*, figure 3. Severe intercrystalline corrosion, as shown, was obtained on the specimen in the oxidizing salt solution.

The cooling curve of a specimen quenched in oil, heated to 98° C, was smooth compared with the one obtained by quenching in water at the same temperature, as is shown in figure 7. Smooth cooling curves were also obtained by cooling specimens in still air and in the blast from an electric fan. Lack of inflections in the curves from the oil and air quenches was interpreted as indicating that the inflections obtained in the curves for the specimens quenched in hot water, figures 5 and 6, were not caused by phase changes in the alloy, but could be attributed to the formation and rupturing of insulating layers of steam on the surfaces of the specimens in the water.

2. DELAYED QUENCHING

In any heat-treatment process, some time is necessarily consumed in transferring the material from the furnace or heating bath to the quenching bath. During this interval the specimens of aluminum alloy used in this investigation cooled at a rate of approximately 10° C/sec while exposed to the air. In handling these small specimens in laboratory equipment, the transfer from furnace to quenching bath was made in 1 second or less. In industrial operations in which a large quantity of material is handled at one time, the delay may be much longer, and before the material enters the quenching

bath, its temperature may fall considerably below that at which it was heat-treated. It was considered important to determine the effect upon the resistance to corrosion of the 24S alloy of varying the heat-treatment procedure by slowly cooling specimens to various temperatures before plunging them into the quenching bath.

Specimens of the three compositions given in table 1 were heated, with thermocouples attached (fig. 1), for 30 minutes at 493° ±5° C, withdrawn from the furnace and permitted to cool to the temperatures given in table 3. They were then quenched in water, the temperature of which had been adjusted to give cooling rates in excess of 1,500° C/sec. After aging, the specimens were immersed in the sodium-chloride–hydrogen-peroxide solution to determine the kind of corrosive attack to which they were susceptible. The temperatures at the instant the specimens entered the quenching bath, and the cooling rates in the bath, were obtained from the cooling curves determined with the string-galvanometer apparatus.

TABLE 3.—*Character of corrosive attack in oxidizing salt solution on specimens of 24S alloy allowed to cool in still air after withdrawal from heating furnace, and then quenched at a rapid rate*

[All specimens given recommended solution heat treatment, 30 minutes, at 493° ±5° C.]

4.05 percent of copper				4.27 percent of copper				4.64 percent of copper			
Quenched when cooled to	Cooling rate		Type of corrosion	Quenched when cooled to	Cooling rate		Type of corrosion	Quenched when cooled to	Cooling rate		Type of corrosion
°C	°C/sec	equiv °F/sec		°C	°C/sec	equiv °F/sec		°C	°C/sec	equiv °F/sec	
430	7,800	14,040	Pitting.	419	8,200	14,760	Pitting.	433	4,550	8,190	Pitting.
418	2,150	3,870	Do.	406	------	------	Do.	430	6,200	11,160	Do.
414	------	------	Do.	405	5,950	10,710	Do.	425	7,900	14,220	Do.
404	4,900	8,820	Do.	399	1,500	2,700	Do.	423	5,100	9,180	Do.
400	4,700	8,460	Do.					414	7,150	12,870	Do.
400	4,500	8,100	Do.					405	7,100	12,780	Do.
								405	5,550	9,990	Do.
396	4,500	8,100	Intercrystalline.	398	9,500	17,100	Intercrystalline.	402	3,100	5,580	Intercrystalline.
379	8,400	15,120	Do.	397	7,600	13,680	Do.	400	7,400	13,320	Do.
375	4,100	7,380	Do.	397	4,650	8,370	Do.	395	7,250	13,050	Do.
				395	2,100	3,780	Do.	392	3,600	6,480	Do.
				394	5,500	9,900	Do.				
				358	5,600	10,080	Do.				

It was demonstrated in this manner (table 3) that 400° C is the minimum temperature at which the rapid cooling of the 24S alloy must begin, if it is to be immune to intercrystalline corrosion. If the specimens entered the quenching bath at temperatures lower than this, no matter how rapid the cooling rates, they were found susceptible to intercrystalline corrosion. Evidently during the time required to cool from the solution temperature to 400° C at the slow rate obtained in still air, the reaction that causes susceptibility to intercrystalline corrosion progresses sufficiently to retain this condition permanently, no matter how rapidly the alloy is cooled thereafter.

Figure 8 shows the change in the type of corrosive attack obtained in specimens of the 24S alloy containing 4.27 percent of copper, by starting the rapid quench after the specimens had cooled in still air from the solution temperature to temperatures just above and just below 400° C. These results indicated also that the minimum tem-

perature at which the rapid cooling must be started is more critical than the minimum cooling rate for the whole interval between the solution temperature and about 120° C.

IV. SUMMARY

1. A study was made of the susceptibility of 24S aluminum alloy sheet to intercrystalline corrosion when cooled from the solution temperature at different rates by quenching in water maintained at different temperatures. Sheets of the alloy, with copper contents of 4.05, 4.27, and 4.64 percent, were made available by the Aluminum Company of America for the study.

2. Temperatures throughout the cooling were determined from photographically recorded time-temperature curves obtained with a string galvanometer and thermocouples that were attached to the specimens. Susceptibility to intercrystalline corrosion was determined by metallographic examination of the heat-treated specimens after they had been immersed in a sodium-chloride–hydrogen-peroxide solution.

3. The rate at which a specimen of 24S alloy must be cooled in order to render it resistant to intercrystalline corrosive attack varies with the copper content of the material. Specimens of the alloy containing 4.64 percent of copper, cooled at rates greater than 1,100° C/sec, were subject to pitting corrosion only, with no traces of intercrystalline corrosion. Specimens of the same material cooled at rates less than 400° C/sec were severely attacked by intercrystalline corrosion in the salt solution. Specimens cooled at intermediate rates showed both pitting and the intercrystalline type of corrosion. Pitting predominated in the specimens cooled at rates approaching the higher one, and as the cooling rate approached the slower of the two above rates, the intercrystalline attack became more severe. Similar results obtained with specimens of 24S alloy having lower copper contents showed that the lower the copper content, the lower the cooling rate required to render the material immune to intercrystalline corrosion.

4. Specimens withdrawn from the heating furnace at the recommended solution temperature of 493° C and permitted to cool in air to a temperature not below 400° C could be quenched so as to be resistant to intercrystalline corrosion; but if they were permitted to cool below this temperature before they were plunged into the quenching bath, they were subject to intercrystalline corrosion in oxidizing salt solution, regardless of the rapidity of the cooling rate in the quenching bath.

WASHINGTON, November 15, 1940.

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DAYTIME PHOTOELECTRIC MEASUREMENT OF CLOUD HEIGHTS

By Maurice K. Laufer and Laurence W. Foskett

ABSTRACT

A photoelectric detector is used in conjunction with a modulated beam of light for the measurement by triangulation of the height of clouds during the daytime. An a-c operated mercury-arc lamp is used to obtain the modulated beam. An electronic "synchronous switch" is used to eliminate the effect of the varying background brightness of the clouds. The shot noise of the phototube, resulting from the relatively high brightness of clouds during the daytime, limits the detection. Dark overcast clouds at an elevation of 9,000 feet have been detected.

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I. INTRODUCTION

A beam of light incident on the base of a cloud is scattered to such an extent that at night the spot produced by a searchlight is generally visible. This phenomenon of scattering of light by water drops has made possible the employment of optical methods for the measurement of cloud heights. Middleton [1] has discussed the use of the ceiling projector [1][2] for night measurements, at which time the angular elevation of the spot can be determined by means of a visual instrument such as an alidade. The height of the cloud is determined from the solution of the vertical triangle formed by the line of sight on the spot, the beam of the projector, and the base line connecting the projector and alidade.

Several years ago Middleton initiated the first work on a photoelectric detector which would extend the use of the ceiling projector to the daytime measurement of cloud heights through the utilization of a modulated beam. Meanwhile F. V. Jones and A. H. Mears, of the United States Weather Bureau, had been considering the same

[1] Middleton also calculated the amount of signal light which is reflected from the spot, and section 3 of his paper applies equally well to daytime and nighttime measurements.

[2] Figures in brackets indicate the literature references at the end of this paper.

solution of the problem when Middleton's work became known to them. The Weather Bureau requested the National Bureau of Standards to develop the necessary equipment for measuring cloud heights in this way. The present paper describes a projector equipped with a mercury-arc lamp operating on alternating current to obtain a modulated beam, and a phototube and amplifier which have been used to detect daytime clouds up to 9,000 feet.

II. MEASURING EQUIPMENT

1. THEORY

Modulating a projected beam so that, after scattering, the light retains its original "identification-tag" was suggested by Tuve [2] as a possible means of studying the upper atmosphere. This suggestion would seem to make it possible to use a phototube and tuned amplifier to sort out completely the identified light from the background light. But the shot noise of the phototube, resulting from the background light, and the other noises associated with the phototube and the amplifier limit the minimum modulated light signal that can be detected [3, 4]. When the background is a daytime cloud, the resulting phototube current may be greater than 10^6 times the signal current which can be obtained from a projector of reasonable size and of moderate power consumption.

Because of the relatively high brightness of daytime clouds, it is possible, if the phototube has very little or no granular noise [3, 5], to design the phototube and amplifier circuit so that all other associated noises are negligible compared with the shot noise of the phototube. This may be done without reducing the ratio of signal to shot noise, and hence the ratio of signal to total noise is increased. The reason is as follows: For any infinitesimal frequency interval, the theoretical ratio of the mean-square shot noise of the phototube to the mean-square thermal noise of the circuit coupling the phototube to the first stage of the amplifier is given by [4]

$$\frac{eI_0Z^2}{2KTR}, \tag{1}$$

where e=charge on the electron, I_0=phototube current, K=Boltzmann's constant, T=absolute temperature, Z=impedance of the coupling circuit for the frequency of the interval, and R=real part of the impedance. (Neglecting tube capacities, $Z=R$ for direct coupling.) Thus, if Z^2/R is made sufficiently large, the shot noise becomes predominant for a given phototube current; and if R is large enough, the thermal noise will exceed the tube noises of the first stage. If, for a narrow band amplifier, Z is considered to be independent of frequency, the signal and the shot noise are proportional to Z. Hence it follows that no sacrifice is made in the ratio of signal to shot noise by increasing Z until the shot noise predominates above the other noises.

The problem of detection is further complicated by the large variations in the background brightness which arise from the motion of the clouds across the beam of the projector, changes in the formation of the clouds, and fluctuations in the amount of daylight incident on the clouds. In order that the total light received will have a detectable modulated component resulting from the projector, the frequency

of modulation of the beam must not be too low; otherwise the random variations in the background light will obliterate the signal.

In continuation of Middleton's initial work, the Canadian Westinghouse Co. used mechanical modulation at a frequency of 400 cycles per second. The precision with which the parts had to be made, and the difficulties encountered with bearing failures because of the high speeds required, indicated that the recently developed high-intensity mercury-arc lamps might prove to be a better means of obtaining a modulated beam. When these lamps are operated on 60-cycle current, the modulation is approximately 95 percent [6] and has a frequency of 120 cycles per second. Although this frequency is less than that used by the Westinghouse Co., it is sufficiently high to overcome the effect of the relatively slow variations of the background light. Recent tests show, however, that this is about the minimum frequency that can be used.

2. PROJECTOR

The projector consists of a 1,000-watt, water-cooled, A–H6 lamp [6], located at the focus of a 24-inch parabolic mirror having a focal length of 10 inches. The dimensions of the mercury arc are approximately 3/50 by 1 inch. The optical axis of the mirror is vertical, and the lamp is mounted horizontally so that the longest dimension of the spot on the base of the cloud is perpendicular to the geometrical plane determined by the beam and the detector. Since the detector must scan the base of the cloud to locate the spot, this arrangement decreases slightly the accuracy with which the optical axis of the detector must coincide with the exact center of the beam during the scanning process. Likewise it is the narrow dimension of the spot that will be traversed, and a more precise determination of the angular elevation results.

3. PHOTOTUBE AND OPTICAL SYSTEM OF THE DETECTOR

Since the signal is proportional to the signal light received, and the shot noise is proportional to the square root of the background light received, the field of view of the detector should include the entire spot, but no more, in order to obtain the maximum signal to noise ratio. Practically, the entire spot is difficult to define. The brightness of the mercury arc [6] is greatest at the center and falls off rapidly at the edges. Imperfections in the mirror of the projector, aberrations, and irregularities in the base of the cloud also tend "to smear" the spot. Probably the most important consideration is the change in the projected area of the spot on a plane perpendicular to the optical axis of the detector as the angular elevation of the spot varies with cloud height.

A diaphragm located at the focus of a lens provides a simple optical system having light-gathering properties and a restricted field of view. For this arrangement the phototube is placed behind the diaphragm. The light rays after passing through the diaphragm are divergent, and if all these rays are to be intercepted by the cathode of the phototube, the focal length of the lens that can be used is determined by the cathode dimensions, the distance between the diaphragm and the cathode, and the aperture of the lens. To a first approximation, the diaphragm opening required to limit the field of view to the spot is equal to the size of the source multiplied by the

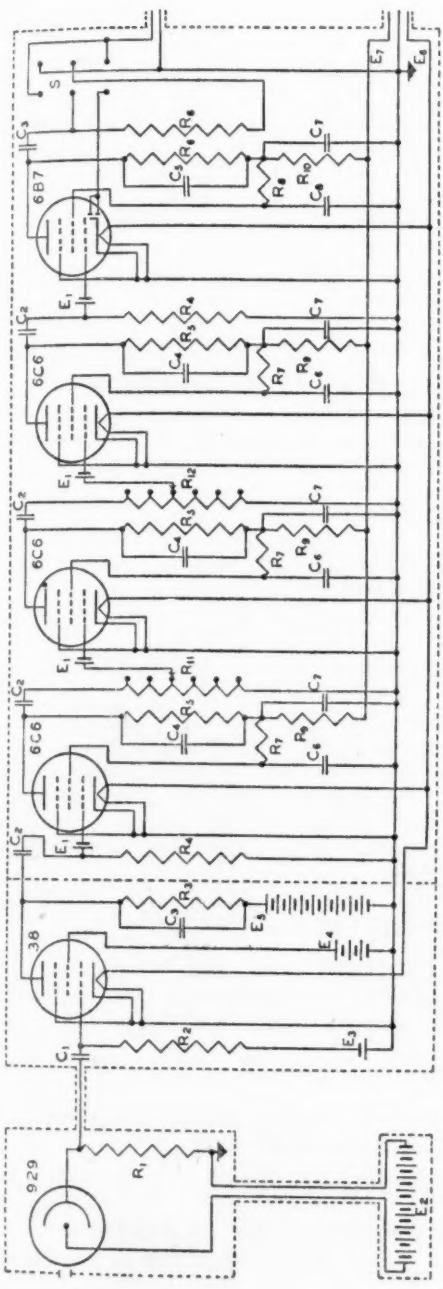
ratio of the focal length of the lens of the detector to the focal length of the mirror in the projector.

In choosing a phototube, one is limited to the vacuum type because of the greater shot noise associated with the gas-filled types [7] for the same phototube current. Barrier-layer photocells cannot be used, because their voltage sensitivity is reduced to practically zero for high flux density. If the phototube is sensitive only to the wavelength interval of the signal light, the effective background light is reduced and hence the signal to noise ratio is increased. The spectral response of the RCA 929 phototube [8] matches the spectral distribution of the light from the A–H6 mercury-arc lamp exceedingly well. Its sensitivity is greater than that of the average gas-filled phototube, and tests have failed to indicate the presence of any granular noise. The dimensions of the cathode are approximately $\frac{7}{8}$ by $\frac{1}{16}$ inch.

The final optical arrangement used consisted of an 8-inch plano-convex glass condensing lens, a diaphragm having a slit opening approximately $\frac{3}{25}$ by $\frac{1}{16}$ inch, with the longest dimension horizontal and the 929 phototube amounted horizontally as close behind the diaphragm as possible. The specified focal length of the lens is 19 inches but, because of the severe chromatic aberration, the diaphragm was located about 17 inches from the convex surface, which corresponds approximately to the focal length for blue light. The long dimension of the diaphragm is equal to the maximum dimension of the cathode surface, and the other dimension was chosen in accordance with the previous discussion. The optical system is mounted so that it may be rotated about a horizontal axis in order to scan the base of the cloud for locating the spot. Phototube currents as high as 25 microamperes have been obtained when the detector is directed at clouds illuminated by direct sunlight, and currents as low as 2 microamperes result from dark overcast daytime clouds. The detector was located about 1,000 feet from the projector.

4. AMPLIFIER

The phototube and 120-cycle, resistance-capacitance tuned amplifier circuit [9, 10] are shown in figure 1. A 5-foot, flexible, shielded cable having a capacitance of 26 $\mu\mu$f per foot connects the phototube to the amplifier. This cable, the blocking capacitor, C_1, the phototube-load resistor, R_1, and the grid-leak resistor, R_2, make up the coupling circuit between the phototube and the first stage. The blocking capacitor is required to prevent the variations in the background light from changing the bias on the first stage. The maximum impedance of the coupling circuit that can be used practically, and consequently the maximum initial signal that can be obtained, is limited by the dynamic grid impedance of the tube of the first stage. The 38-type tube has a high dynamic grid impedance, since the grid current for the voltages used is less than 10^{-11} ampere [3]. The choice of this tube permits the use of a 10^8-ohm grid leak, R_2. Under these operating conditions the grid current shot noise is negligible. The values of the component parts of the coupling circuit fulfill the conditions required by eq 1 for shot noise predominance and also sharpen the tuning of the amplifier. It was found necessary to use for C_1 a capacitor having a leakage resistance greater than 10^{12} ohms to keep the grid bias constant. At 79 and 180 cycles the amplification of the amplifier is 0.707 times the maximum amplification at 120 cycles.



	Microfarads
C_1	0.0000125
C_2	.0025
C_3	.002
C_4	.0055
C_5	.004
C_6	.1
C_7	2.0
C_8	0.25

	Volts
E_1	1.0
E_2	270
E_3	1.5
E_4	4.5
E_5	45
E_6	16
E_7	135

	Megohms
R_1	10
R_2	100
R_3	1
R_4	5
R_5	0.25
R_6	.5
R_7	4.3
R_8	6
R_9	1.4
R_{10}	0.08
R_{11}	5.14
R_{12}	5.05

S=DP–DT switch

FIGURE 1.—*Wiring diagram of phototube and 120-cycle, resistance, capacitance tuned amplifier having the following circuit constants:*

The diode part of the 6B7 tube is used in conjunction with a d-c meter for checking the operation of the amplifier, for making noise measurements, and for studying the effects of the varying background light. As used, the amplifier and phototube are operated with batteries. Since the fundamental ripple frequency of a full-wave rectifier is 120 cycles, a-c operation may prove to be troublesome. In this connection, the total plate and screen current for the five stages is less than 1 milliampere. It is believed that this small current will make possible the design of an a-c power supply for the plate and screen voltages by using a resistance-capacitance filter to reduce the 120-cycle ripple considerably below the signal. A power supply of this type has been used satisfactorily for the phototube.

5. OUTPUT CIRCUIT AND METER

According to Johnson [10, 3], for an amplifier having a uniform amplification between the frequencies f_1 and f_2 and zero elsewhere, the smallest steady signal, E_s, that can be detected in the presence of a statistical noise voltage, E_n, is given by

$$E_s=(5\lambda/(f_2-f_1))^{1/2}E_n, \tag{2}$$

where λ is the decay constant of the output meter. Also, for direct coupling of the phototube to the amplifier, the input noise level of the coupling circuit and the amplifier is given by

$$\overline{E_n^2}=4KT\{(1/2\pi C)(\tan^{-1}[2\pi RC(f_2-f_1)/(1+4\pi^2R^2C^2f_1f_2)] (1+19.4I_gR)+R_t(f_2-f_1)\} \tag{3}$$

where K=Boltzmann's constant, T= absolute temperature, C= sum of static and dynamic input-capacities, R=the phototube load resistance, I_g=sum of the phototube current and the grid current from the input tube, and R_t=equivalent noise resistance of the input tube. Replacing the arc-tangent by its argument (which increases the value for the noise slightly),

$$\overline{E_n^2}=4KT(f_2-f_1)[R_t+R(1+19.4I_gR)/(1+4\pi^2R^2C^2f_1f_2)]. \tag{4}$$

For $R=10^7$ ohms, $C=1.3\times10^{-10}$ farad, and $I_g\geq2\times10^{-6}$ ampere, $19.4\ I_gR>>1$ and $19.4\ I_gR^2/(1+4\pi^2R^2C^2f_1f_2)>>R_t$, where R_t is about 5×10^4 ohms [3]. Hence eq 4 may be written as

$$\overline{E_n^2}=77.6KT(f_2-f_1)I_gR^2/(1+4\pi^2R^2C^2f_1f_2) \tag{5}$$

The signal-voltage squared, E_s^2, resulting from the root-mean-square signal-current, I_s, in the phototube, is given by

$$E_s^2=I_s^2R^2/(1+4\pi^2f_0^2R^2C^2), \tag{6}$$

where f_0 is the modulation frequency of the signal light.

If the percentage band width of the amplifier is comparatively small, that is, if

$$(f_2-f_1)/(f_1f_2)^{1/2}\lessapprox1 \tag{7}$$

then

$$f_0^2\doteq f_1f_2 \tag{8}$$

and the ratio of signal to noise from eq 5 and 6 is given by

$$E_s/E_n = I_s/[(f_2 - f_1)(77.6KTI_g)]^{1/2}. \quad (9)$$

Equating E_s/E_n from eq 2 and 9, the minimum root-mean-square signal-current that can be detected is given by

$$I_{s_{min}} = (388\lambda KTI_g)^{1/2}. \quad (10)$$

This equation, based on Johnson's original eq 2, 7, and 8 is contrary to the accepted idea, namely, that the minimum detectable signal current is proportional to the square root of the band width [4, 7, 11, 12]. As pointed out by Johnson, eq 10 shows that $I_{s_{min}}$ does not depend on the band width, if the band width is sufficiently narrow to satisfy eq 7, but depends on the decay constant of the output meter; that is, the minimum detectable signal is inversely proportional to the square root of the period of the output meter.

If it is assumed that the band width of the amplifier of figure 1 is 101 cycles (that is, $f_1 = 79$ c/s and $f_2 = 180$ c/s, the frequencies at which the amplification is 0.707 times the amplification at 120 c/s), eq 7 is satisfied. Furthermore, the approximations that must be made to obtain eq 5 when using the complex impedance for the coupling circuit introduce an error which is the same order of magnitude as that used above. The case for direct coupling is given for simplicity.

The above discussion is valid for noises which are constant except for statistical fluctuations. But during the time the base of the cloud is being scanned to find the spot, the output current will be varying in general by an amount greater than that which will result when the spot is located even if a long-period meter is used to damp out almost completely the statistical fluctuations. This results from the variations in the background brightness of the clouds as stated before, and even more so from the change in brightness from cloud to cloud as the scanning is made. If, however, a 120-cycle synchronous commutator, which is phased with the signal, is introduced between the amplifier and the meter, the average output current in the absence of the signal will be zero because of the random phase of the shot noise. Thus the variations in brightness will not give a false indication of the signal, since the same average zero reading will be obtained for the shot noise whether the average phototube current is 25 microamperes or 2 microamperes. A second advantage results because effectively an increased scale length can be used. Normally the shot noise would produce an average reading 10 to 100 or even 1,000 times that resulting from the signal, but by reducing the average noise-output current to zero the entire scale of the meter can be used for indicating the signal.

There is a practical limit for the maximum period of the meter that can be used. If the scanning is made at a reasonable rate and if the period of the meter is too long, the spot will be passed without obtaining a noticeable indication. A period greater than 1 minute has been found to be unsatisfactory.

An "electronic switching-circuit," which performs the same function as a synchronous commutator, is shown in figure 2. The rectified, but nonfiltered, output of the transformer, T_1, is the plate supply for the 6J7 tube. If the resistance, R_4, is correctly adjusted, the plate current consists of square-topped pulses which are maintained for

approximately 1/240 second. The time-average of the IR drop across the plate resistor, R_3, can be obtained from the potential across the capacitor, C_2, and this potential is reasonably steady because of the long period of the series circuit consisting of R_2 and C_2. Furthermore, the magnitude of this potential is almost completely independent of the amplified shot noise from the amplifier because of the random phase as in the case of a synchronous commutator. But when a sustained and phased 120-cycle signal is added, the potential across C_2 increases or decreases depending on whether the positive or negative

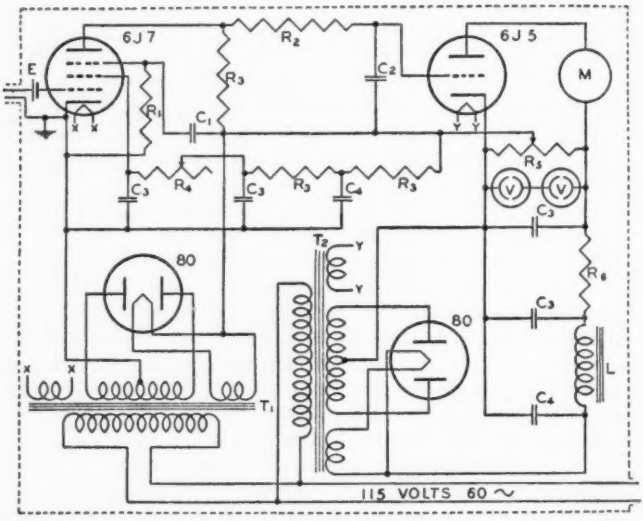


FIGURE 2.—*Wiring diagram of output circuit and meter having the following circuit constants:*

	Microfarads
C_1	0.0001
C_2	4
C_3	40
C_4	4

	Megohms
R_1	20
R_2	3
R_3	0.1
R_4	.1
R_5	.2
R_6	.003

T_1=830 volts a-c, CT.
T_2=700 volts a-c, CT.
E=1.25 volts (Mallory grid bias cell).
L=15 henries at 85 milliamperes.
M=0 to 10, d-c milliammeter.
V=VR-150 voltage regulator tube.

portion of the signal is present during the time the plate current is flowing. A previous switching circuit contained a phase-shifting element in front of the primary of the transformer, T_1; but it so happens that it is not needed, because the positive portion of the signal is correctly phased to within a few degrees when the transformer for the mercury-arc lamp in the projector is connected to the same alternating-current supply. The remainder of the circuit shown in figure 2 is essentially a vacuum-tube voltmeter, which is used to indicate the change in the potential across C_2.

In practice, the potentiometer, R_5, is adjusted to give a plate current of about 7 milliamperes. In general, this current will be fluctuating by about 0.5 milliampere. If, during the scanning process to find the spot, the current drops to less than 6 milliamperes,

one can be sure that the spot has been located. When the signal is so weak that the decreased reading fluctuates about this point (6 milliamperes), the detection can be verified by repeating the traverse to make sure that a spurious indication had not been obtained.

III. RESULTS

During the daytime, dark overcast clouds at an elevation of 9,000 feet have been readily detected with the equipment described. For cumulus clouds illuminated by direct sunlight and having elevations up to 4,000 feet, the detection is positive.

In the laboratory, measurements have been made to determine the minimum signal which can be detected with certainty in the presence of shot noise. Battery-operated incandescent lamps were used to simulate the background light and were arranged to give a phototube current of 5 microamperes. A sector disk driven by a synchronous motor was placed between a small flashlight lamp and the phototube to obtain a 120-cycle chopped signal. The flashlight lamp was located finally about 80 feet from the phototube. The resulting signal light was about 5×10^{-7} times the background light and according to eq 10 the signal current was less than 2 times the theoretical minimum.

IV. DISCUSSION

There are several possibilities for increasing the sensitivity of the equipment. Among the most obvious are the use of a larger mirror in the projector to give a more intense beam of light and the use of a larger lens in the detector. The latter possibility would increase the flux density on the cathode of the phototube to such an extent that it might be excessive and destroy the photosensitive surface. If the signal could be restricted to a single wavelength, it is conceivable that the background light could be reduced to a negligible factor by using filters which transmit the signal light and only a very small part of the background spectrum. The high-intensity mercury-arc lamp is the only practical source of high brightness having a considerable percentage of its energy emitted in narrow wavelength intervals. But to take advantage of this, one requires filters which have narrower transmission bands than are now available. Many filter combinations have been tried, but a net loss was obtained rather than a gain in the signal to noise ratio, because the signal is proportional to the transmission of the filter whereas the shot noise resulting from the background light is proportional to the square root of the transmission.[2]

The authors thank E. A. Johnson, of the Department of Terrestrial Magnetism, Carnegie Institution of Washington, for several helpful discussions and for granting them the permission to read reference [3] in manuscript form.

[2] Let I_s and I_g be the phototube currents resulting from the signal light and the background light, respectively. Then the ratio of signal to noise, E_s/E_n, may be written as $kI_s/I_g^{1/2}$. If a filter is introduced, the ratio of signal to noise becomes $kI_sT_s/(I_gT_g)^{1/2}$, where T_s and T_g are the respective transmission factors based on the spectral response of the phototube for the signal light and for the background light. Hence $T_s/T_g^{1/2}$ must be greater than unity in order to increase the signal to noise ratio.

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RESEARCH PAPER RP1380

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A MANOMETRIC GAS ANALYSIS APPARATUS

By Martin Shepherd and E. O. Sperling

ABSTRACT

The apparatus here described is designed for the analysis of gas mixtures under the following conditions:

(1) When the volume of the sample is small—about 5 to 0.5 ml—and a reasonably accurate analysis is desired.

(2) When the volume of the sample is of the order of magnitude of 0.2 to 0.1 ml, and an approximate microanalysis is desired.

(3) When the gases to be analyzed are dissolved in some liquid from which they must be liberated prior to analysis, as in the analysis of blood gases.

The apparatus is adapted for use with the admirable procedures of Van Slyke. The present design eliminates the two principal errors inherent in the Van Slyke apparatus, as well as the mechanical shaking, which some have found to be an objectionable feature. By extending the apparatus here described, analysis by combustion may be performed, and greater operating conveniences achieved if desired.

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I. INTRODUCTION

In the field of gas analysis which lies between the strictly micromethods and the usual macromethods, and which includes the analysis of gases dissolved in liquids such as blood, the work of Van Slyke is outstanding. The Van Slyke methods have been described so comprehensively that the text[1] can be recommended as a standard reference on this particular type of gas analysis.

In 1934, several errors inherent in the Van Slyke apparatus were discussed and experimentally demonstrated.[2] At that time, a modification of the Van Slyke apparatus and procedure was suggested as follows:

"The Van Slyke apparatus may be modified by placing two parallel chambers in the water jacket, one to function as an extraction and reaction chamber, the other as a fixed volume burette.

"This procedure would possess several additional advantages. It would always be possible to avoid redissolving some portion of an

[1] John P. Peters and Donald D. Van Slyke, Quantitative Clinical Chemistry, Vol. 2 (The Williams and Wilkins Co., Baltimore, Md., 1932).

[2] Martin Shepherd, *Significant vapor pressure considerations of the Van Slyke manometric methods of gas analysis*, BS J. Research **12**, 551 (1934) RP680.

extracted gas sample, for which the correction is troublesome and not always satisfactory. This could be achieved by transferring the liberated gas from the extraction chamber to the burette under the pressure at which extraction occurred. Each reagent could be freed from gas in the apparatus before bringing the gas mixture into the reaction chamber. Physical solution of components of the gas mixture in the reagent employed could be reduced for macroanalyses and almost avoided for microanalyses. The various fractions of the gas mixture would always be accurately measured under the same condition of humidity. The measurement of gas volumes (or pressures) would always be made by adjusting a clean mercury meniscus to a reference mark, thus avoiding obscure solution menisci. Solutions from which gas samples were extracted, as well as reagents subsequently employed, could be expelled from the extraction chamber at each step of the analysis. This technique would permit straightforward, clean reactions with no interference, either chemical or physical, from accumulating mixtures of various liquids that have already served their purpose in the analysis. Reagents of any desired strength could be employed. There would be no hydrostatic pressure to correct for, and the *c correction*, as well as all others applied by Van Slyke, would merely resolve into a simple correction for the change of pressure of the gas and saturated water vapor with any change of temperature which might occur during the analysis."

Shortly thereafter an apparatus which realized all of the advantages mentioned above was constructed. The use of this apparatus in our laboratory has been limited during the past 6 years, and other projects have prevented its further development along lines suggested in what follows. Its possibilities, accordingly, have not been thoroughly explored, but enough experimental work has been done to indicate its value and warrant describing it in its present form.

II. DESCRIPTION OF APPARATUS AND PROCEDURES

The construction of the apparatus is illustrated in the drawing (fig. 1) and photograph (fig. 2). The essential feature is the inclusion of an extraction and reaction chamber, *R*, which is separate from the constant-volume burette, *B*. The Van Slyke apparatus has only a vessel corresponding to *B*.

For those not familiar with this type of apparatus, a very brief outline of the general procedure to be used with the Van Slyke and the new apparatus will serve to clear the picture and to point out the reasons for the new deisgn.

With the Van Slyke unit, the procedure is as follows: Blood to be outgassed (or a sample of free gas) is introduced over mercury into the single vessel corresponding to *B*. The mercury is then dropped to a level corresponding to the 50 ml mark of figure 1, and the evolved gas is collected in the partial Torricellian vacuum above. The gas is then measured by raising the mercury back to a level corresponding to the 2 ml or 0.5 ml mark and noting the pressure on the attached manometer. Some gas will redissolve in any liquid present during this procedure. Next, the reagents are admitted, one at a time, to absorb various constituents of the freed gas. With the addition of each reagent, a correction for hydrostatic head must be made. Furthermore, a correction for the lowering of vapor pressure is neces-

sary each time; and, as this is prescribed by Van Slyke, a change in temperature may introduce further error in this correction.[3] In addition, the accumulating reagents (which must be selected with reference to their chemical behavior with each other as well as with the gases) cause increasingly greater errors of physical solubility, which must be accounted for. Sometimes these reagents make difficult the adjustment of the confining meniscus.

This procedure should be compared with the general one used with the new apparatus. Here, if a sample of blood or liquid containing dissolved gases is to be examined, it is introduced into *R* and there outgassed. The *gas* only is then transferred, at the lowest pressure existing during outgassing, to the clean burette, *B*, and there, isolated from the liquid, it is measured while saturated with respect to water vapor only. The blood or other substance is then expelled from *R*, the first analytical reagent is introduced and outgassed directly in *R*, the gas liberated from this reagent is discarded, and then the gas in *B* is transferred to *R* for the reaction. After the absorption, the residual gas is transferred back to *B* at the lowest pressure existing during absorption, and again measured while saturated with respect to water vapor only. The two volumes so measured compare directly with respect to saturation. No gas has been absorbed by the original liquid, or by the reagent. Furthermore, no gas has been given off from the reagent during the actual absorption.

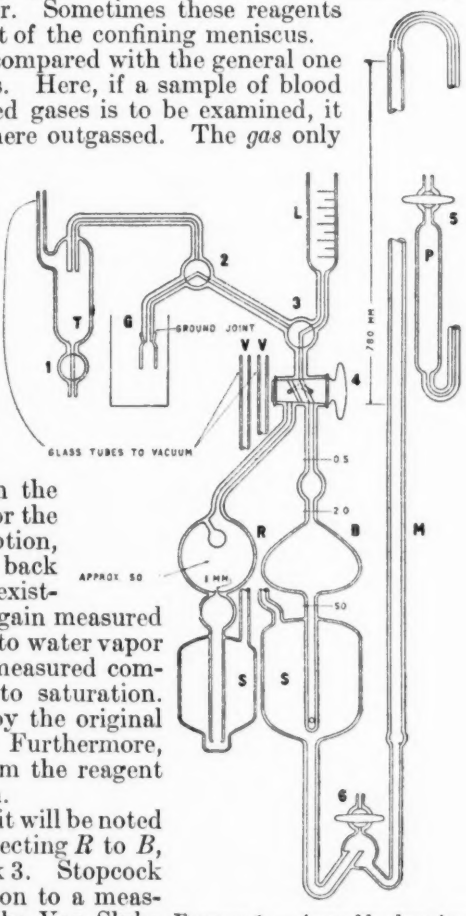

FIGURE 1.—*Assembly drawing of manometric gas analysis apparatus.*

Referring again to figure 1, it will be noted that stopcock 4 permits connecting *R* to *B*, and either *R* or *B* to stopcock 3. Stopcock 3, in turn, provides connection to a measuring tube *L* (a feature of the Van Slyke design which has been retained) or to stopcock 2. Finally, stopcock 2 offers connection to a trap used to collect and discard liquids from *R*, and to a small bell, *G*, which may be used to collect small samples of gas for analysis when this bell is immersed in mercury.

With this arrangement, liquids to be outgassed are measured in *L* and transferred therefrom to *R*, the various reagents are introduced by the same route, exactly as prescribed by Van Slyke, a procedure which is objectionable because of the difficulty of maintaining the lubricant of stockcock 4 in good condition under this treatment. For this reason, a later modification, not shown in figure 1, has been added.

[3] See footnote 2 for complete discussion of this "c correction."

This is a capillary delivery tube leading from a reservoir (located next to T) directly into the middle bulb of *R* by means of a ring seal. The reservoir is provided with a vacuum connection at the top, and the reagent, in considerable quantity, can be outgassed in this reservoir. This eliminates one step in the analysis, and provides a reserve supply of gas-free reagent. As many of these reservoirs as there are required reagents, connected by a suitable manifold for delivery of liquids from the bottom and another for evacuation from the top, would prove a great convenience. This arrangement also prevents fouling of the lubricant of stopcock 4, which *must never leak*.

In the introduction of blood or other liquids for outgassing, sampling in a good hypodermic syringe is suggested. The male member of an interchangeable ground-glass joint should be attached to the outlet of a syringe modified for this purpose. The corresponding female member is sealed into the bottom of the tube, *L*.

In the Van Slyke model, gas is driven from solution and the reactions are performed with the aid of mechanical shaking. We have had no experience with the actual device used, but we understand from others that it is objectionable, and sometimes causes breakage. In the present model, the liquid to be outgassed, or the reagent to be brought into intimate contact with the gases, is transferred over mercury to the smaller middle bulb of *R*, and then forced back through the 1-mm nozzle at the top of this bulb at rates great enough to cause it to squirt with considerable force against the spherical baffle directly above the nozzle. At the low pressures existing within *R*, this procedure very effectively (indeed almost spectacularly) breaks up the liquid and brings it into intimate contact with all of the space in *R*.

The mechanics of this system may be readily understood when it is pointed out that the bulbs *S*–*S* at the bottom of both *R* and *B* are filled with mercury, and the two connections from the tops of these bulbs, *V*–*V*, each terminate in a tee, to both arms of which are affixed needle valves with vacuum-tight packings (Stimson type). One of each pair of these valves connects to a vacuum line, the other to the air. Opening the valve to vacuum lowers the mercury in *S*–*S*; opening to air forces the mercury into *R* and *B*, since the pressures within *R* and *B* are low. By means of the needle valves for control, mercury can be raised or lowered as slowly or rapidly as desired. Thus, the meniscus in *B* can be very nicely adjusted to the reference marks, or the liquid in *R* forced rapidly through the nozzle. Once learned, this operation is very conveniently performed. We have, however, watched this manipulation fail repeatedly because of the apparent inability of an operator to acquire the necessary skill. Therefore, the following modification is suggested for those who may prefer it. The lower ends of *S*–*S* may be terminated in tubes to which control stopcocks [4] are sealed. To these cocks, ordinary leveling bulbs may be connected by means of rubber nitrometer tubing. Ring supports for these bulbs, at the levels of stopcocks 3 and 6, will permit adjustment of the mercury levels so that the necessary operations can be performed. The leveling bulbs need not be held in the hand, but instead, the flow of mercury can be regulated by the control cocks with the bulbs in one of the two fixed positions. Traps should be interposed between the leveling bulbs and the two vessels *R* and *B*, so that no air can be carried into the apparatus.

[4] Martin Shepherd, *A simple control stopcock for gas analysis apparatus*, BS J. Research **4**,23 (1930) RP130

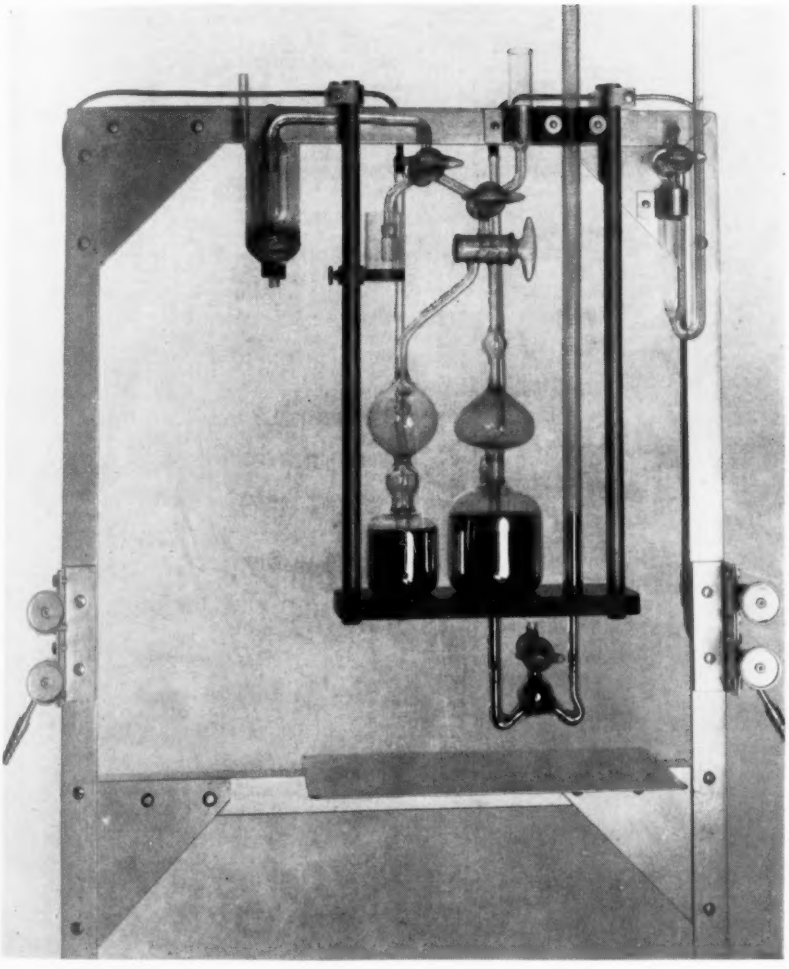

FIGURE 2.—*Manometric apparatus.*

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The burette, B, is calibrated as shown (fig. 1). For approximate microanalysis, lesser volumes should be included in this calibration. Blackened copper collars are affixed to the stems at the etched lines, since the adjustment of a mercury meniscus to the lower edge of such a collar, in the manner in which a barometer is read, can be made with greater reproducibility than adjustment to an etched mark.[5]

The manometer, M, registers the pressures obtained within B. This may be provided with a sliding scale with stops at the three positions corresponding to the levels of the three reference marks on B; or the scale may be engraved on the tube of M, and the three readings of this fixed scale, which correspond to the reference marks on B, can be determined with a suitable telescope. The stopcock affixed to the top of the Van Slyke manometer has been eliminated in favor of the considerably more certain arrangement shown (fig. 2). This is a standard form in which the vacuum is obtained by forcing mercury around the capillary at the top of M and down into the reservoir, P. Vacuum applied at stopcock 5, and pressure through stopcocks 2 and 4 to B, will fill the manometer. Opening cock 5 to the air will complete the operation. The lower entrance to M is guarded by trap 6, from which accumulated gas may be removed via the stopcock there placed.

The whole unit, up to the level of stopcock 4, is immersed in a water jacket. A good grade of museum jar is well suited for this purpose. The method of mounting is shown in the photograph. The jar rests upon the metal shelf shown below the trap at stockcock 6. The needle valves for control of mercury flow are conveniently placed to the right and left on the supporting frame.

After the discussion which has preceded, no detailed schedule of operation is necessary. For the actual technique and procedures involved, the remarkably complete account of Van Slyke should be consulted, and modified only as indicated in the following section.

III. PRECAUTIONS IN OPERATING THE APPARATUS

Three characteristics of the new apparatus should be mentioned in order to avoid trouble during the initial stages of manipulation.

It must be remembered that, while the pressures in R and B during an analysis are low, they are nevertheless above atmospheric. It is therefore possible, by injudicious and too abrupt manipulation of the controlling needle valves, to reduce the pressures above the mercury in the reservoirs S–S to a point where gas and liquid in R, or gas in B, will be drawn down the tubes and around the mercury seals into S–S. This is easily enough avoided, and a little experience will suffice to eliminate the danger.

Sometimes the spherical baffle in R does not prevent the formation of slugs of liquid in the capillary connecting this bulb to stopcock 4. If these form, they must be removed before transferring gas from R to B—otherwise the whole object of the new design will be defeated. These slugs of liquid can be removed by adjusting the level of liquid in R to the lowest point, and then slowly admitting mercury from L through 3 and 4 to R.

Once in a while, if the mercury and liquid above it have been too abruptly lowered in R, a bubble of vapor forms beneath the nozzle

[5] E. R. Weaver and Martin Shepherd, *A burette for the accurate measurement of gas volumes without gas connection to a compensator*, Sci. Pap. BS **22**, 375 (1927) S509.

and prevents drainage of some of the liquid (not mercury) into the small middle reservoir. The only remedy for this difficulty is to force the mercury back through the nozzle and repeat the operation of lowering the liquid, using a more gentle approach. This difficulty has been experienced occasionally with a 1-mm nozzle, but not with a 2-mm nozzle. The larger nozzle does not produce the fine spray and complete breaking up of the liquid that was obtained with the smaller one. The optimum size has not been determined, but the smaller of the two sizes tried seems preferable.

In addition to the above precautions, the prospective user should have in mind the general volumetric limitations of an apparatus of this type. In this particular model, when gas is transferred from *R* to *B*, it should be remembered that liquid from *R* must not enter *B*. This means that the reagent in *R* must be raised only to a point level with the entrance of the V-bore of cock 4. The bore of this cock then contains some of the gas that should be measured in the fixed volume burette, *B*. A slight error is thereby introduced. This may be calculated for the worst cases:

(1) the volume of the bore is 0.05 ml. (It could be made smaller.)

(2) The pressure of a gas in *B* adjusted to any reference mark rarely exceeds 600 mm.

(3) The percentage errors will be highest when small volumes are measured. Therefore, assume 0.5 ml of gas at 600-mm pressure. When this gas is transferred between *R* and *B*, the pressure existing in the V-bore of cock 4, which is the pressure of the gas trapped therein, is 6 mm. Its volume at 600 mm would therefore be 0.0005 ml. This is 0.1 percent of the total gas volume.

(4) Since, with each measurement of a volume, the amount of gas trapped is decreased in proportion to the amount absorbed, the resulting error in comparing any two constituents is <0.1 percent of the mixture, and the overall error from this source, if all constituents were removed, would be 0.1 percent.

This feature of design is therefore not objectionable. Much more serious is the fact that the amount of water above and below the mercury in *B* may vary. In practice a film of water should be kept upon the walls of *B*, but water must not obscure the mercury meniscus. If water collects above the mercury, the "fixed" volume will have changed; and the amount of this change may be expected to shift slightly but significantly during the analysis. This difficulty is somewhat more aggravated in the Van Slyke model, but is sufficiently troublesome in the present one to prevent the use of the apparatus for accurate work with very small volumes. The same thing is true for most micro gas analysis apparatus. If high accuracy is required, one must use considerably more complicated apparatus, and procedures, involving the transfer of gases by condensation or freezing to negligible vapor pressures, or by mercury displacement pumps, and the measurement of gas in the dry state.

WASHINGTON, November 14, 1940.

U. S. Department of Commerce National Bureau of Standards

RESEARCH PAPER RP1381

Part of Journal of Research of the National Bureau of Standards, Volume 26, April 1941

AN APPARATUS FOR THE ABSORPTION OR GRAVIMETRIC DETERMINATION OF CONSTITUENTS OF A GAS MIXTURE

By Martin Shepherd and Harry W. Bailey

ABSTRACT

The apparatus here described eliminates many of the undesirable features of certain types of "absorption trains" which are intended to remove constituents from gas mixtures or to determine them gravimetrically. It is adaptable to many uses, since auxiliary units, such as combustion apparatus, may be connected by means of interchangeable joints.

One of the commonest procedures associated with gas chemistry is the removal of one or more constituents of a gas mixture by reaction with solid or liquid reagents, for purification or for the gravimetric determination of absorbed constituents. The various types of apparatus used to conduct such reactions have been referred to as absorption or purification trains, or combustion trains (as employed in the analysis of steels, rocks, organic compounds, etc.). These so-called "trains" are usually constructed to meet the requirements of a particular experiment, and more often than not have a makeshift appearance. Even when they are assembled for more permanent use, little attention is given to convenience, and awkwardness has gradually become a characteristic of such apparatus.

When gases or vapors are to be absorbed and the amount of absorbed material is to be determined by increase in weight, the apparatus should have the following desirable features:

(1) The absorbers should be connected by means of well-ground joints, and not by rubber connections that will admit significant amounts of water or permit the loss of such gases as carbon dioxide.

(2) The connections should be made in such a way that the absorbers can be removed and replaced without disassembling the whole unit.

(3) The connections should be arranged so that other units, such as combustion tubes, can be inserted to increase the usefulness of the apparatus.

(4) The absorbers themselves should be as light as possible, so that their weight is not large in proportion to the weight of the absorbing reagent they contain.

(5) The absorbers should be easy to mount on the balance pan.

(6) The shape and finish of the outside surface of the absorbers should be such that they may be easily cleaned, and particularly so with respect to lubricant applied to the connections.

(7) The absorbers should be designed so that they may be readily emptied and cleaned, and easily charged with reagent without fouling the grinding.

(8) The absorbers should bring the gas into intimate contact with the reagents, and, if possible, be suitable for both solid and liquid reagents.

(9) The absorbers must be provided with a suitable means of opening and closing.

(10) The unit should be mobile, so that it can be taken—in one piece—from job to job.

The apparatus shown in figure 1 possesses all of these desirable features. A detailed drawing of the absorber is given in figure 2.

The absorber is blown from light-wall tubing. While the size may be varied for special purposes, that shown is suitable for general analytical work, and weighs approximately 14 g. There are two parts: a ground cap, 1, bearing the side arms 4–4; and the tube, 7,

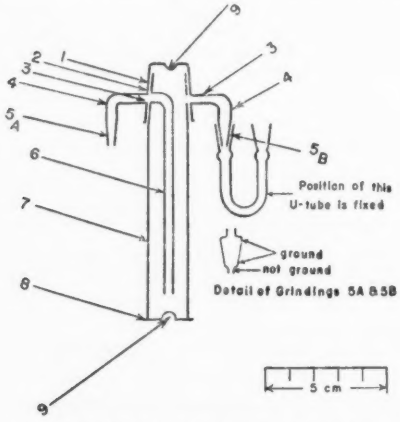

FIGURE 2.—*Detail of absorber for gravimetric apparatus.*

See text for explanation.

whose top is ground at 2 to correspond to the grinding of the cap. These grindings are standard interchangeable ones. The grindings at the end of the inlet and outlet arms, *5A* and *5B*, are also interchangeable. The bores at 3–3 are 4 mm in diameter. The inlet tube, 6, brings the gas to the bottom of the absorber and permits use with solid or liquid reagents. The bottom of the absorber, 8, is flattended so that it rests securely "on its own feet" on the balance pan or elsewhere. The cap and tube are dimpled at 9–9 to permit supporting in the manner to be described.

In charging the absorber, the reagent does not come into contact with the top grinding as is the case with the tubes generally used. The grindings *5A* and *5B* are male, and lubricant is accordingly easily dissolved from them before weighings are made. The lower portions of these grindings are cut in and left unground, so that lubricant does not tend to work into the tube—an old suggestion of E. W. Morley. The grindings, *5A* and *5B*, are pointed *vertically downward*, so that they can be *lifted directly out* of corresponding female members. The female members are connected by a U-tube, which is *permanently*

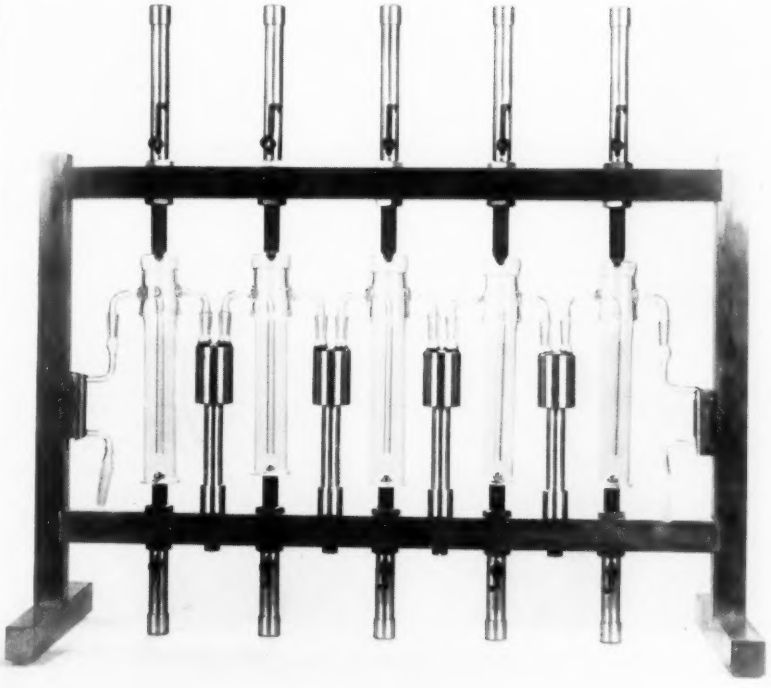

FIGURE 1.—*Gravimetric apparatus.*

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fixed in position. Thus, an absorber is removed from the train by merely lifting it out, and is replaced as easily. The generally employed method of disconnecting the absorbers by pulling apart horizontally—which means taking the whole apparatus apart—is excessively awkward. The absorbers are opened or closed by rotating the *lower* member, 7, and not the cap or separate plugs as is the general practice.

Referring to the photograph (fig. 1), it will be seen that the U-tubes which terminate in the female grindings that provide the fixed connections to the absorbers, are mounted (in a cement) within the four brass cups and the two end clamps of the supporting frame. This mounting is done once for all by joining the whole train together with a low-melting thermoplastic, placing the whole in position on the frame, and pouring the cement to hold the U-connections permanently into the brass cups. Then the thermoplastic is dissolved from the grindings, and thereafter is replaced by lubricant.

It is necessary to hold the grindings of cap and tube (1 and 2), and of the side arms and U- connection (*5A* and *5B*) snugly together, since gas pressure within the train might force them apart. This is done by fiber plungers tapered at the end and bearing in the dimples 9–9 at top and bottom of the absorber. Compression is applied to these plungers by means of springs encased in the sets of five brass tubes at the top and bottom of the frame. The upper set of springs applies slightly more tension than the lower set. Thus the two types of grindings are held seated. The plungers are raised and lowered by small handles which may be locked in position to hold them away from the absorbers.

Since all grindings are interchangeable, convenient connection to other apparatus, gas supply, metering devices, etc., is available. In addition, one or more absorbers may be replaced by other units, such as combustion tubes. The apparatus can thus be adapted to many uses.

WASHINGTON, November 14, 1940.

U. S. Department of Commerce National Bureau of Standards

RESEARCH PAPER RP1382

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MODIFICATIONS OF APPARATUS FOR VOLUMETRIC GAS ANALYSIS

By Martin Shepherd

ABSTRACT

This paper describes modifications of the volumetric gas analysis apparatus originally described in this journal, volume 6, page 121 (1931), Research Paper 266. These modifications include new pipettes, particularly a new type designed for use with small amounts of solid or liquid reagents. The new equipment is not intended to replace, but rather to supplement the apparatus previously reported.

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During the course of experimental studies in gas analysis, the apparatus, which was first described in this journal in 1931, has been modified in some respects. These modifications are described herein. They may be regarded as optional and are not offered as required changes of the original apparatus, unless otherwise noted. They seem of sufficient interest to report at this time, especially in view of the fact that it is unlikely that a general revision of the design of the original volumetric unit will be described for some time.

It will be convenient, in the interest of economy of space, to refer to the previous description of the apparatus. This will be done by citing the number of the paper, RP266. For the further convenience of those who are familiar with this publication, the various modifications will be discussed in the same order as that in which the parts were described in the original paper.

I. THE BURETTE

Most of the users of this apparatus, including men in our own laboratory, have found it convenient to supply the nitrogen used as a diluent during analysis by treating air with the pyrogallol solution. This automatically eliminates the necessity for the atmospheric bore of the stopcock affixed to the top of the burette (RP266, p. 123, fig. 1, stopcock 2). This bore had better be omitted unless the user particularly desires it.

Analysts have long wished for a burette to which no correction need be applied, and some time ago the advent of the KPG precision-bore tubing, made by Schott u. Genossen, offered the possibility of fulfilling this wish. It should be possible to construct such a burette at no great additional cost, since the actual *units* employed in its calibration do not matter, so long as they compare strictly one with another. In addition, the use of diluent nitrogen eliminates the necessity for measuring volumes smaller than approximately 5 ml, so that the destruction of the uniform bore at the very top of the burette where the cock must be sealed on is a matter of no concern. With these two facts in mind, the calibration should offer very attractive possibilities to the manufacturer. Instead of locating at least 5 and preferably 10 points along the tube by repeated weighings of mercury, and *then* setting up the dividing machine with the proper ratios for each of these measured intervals, the machine could merely be set to rule equal intervals of, say, 1.5 mm each along the length of a precision-bore tube. The only point which would have to be located would be one near the top of the tube, so that the first line engraved would leave above it a whole number of whatever the unit happened to be. If the same size precision bore were always used, as it should be, the location of this one point could be easily accomplished by means of an auxiliary volumetric apparatus already designed. This would so greatly reduce the cost of calibration and engraving that the additional cost of the precision-bore tube would be nearly canceled. The analyst when relieved of the irksome obligation of applying corrections would, without doubt, be glad to pay the slight premium that might be necessary to obtain this advantage.

We had one such burette constructed from tubing of foreign manufacture. The bore was so uniform that any 10-ml interval throughout its length equalled any other 10-ml interval within ± 0.01 ml. This tubing is not generally available, and a source of tubing of sufficiently uniform bore has not been located in this country. As worldly matters are disposed at present, we hope that an ambitious American manufacturer will undertake the production of this item.

II. BURETTE ILLUMINATOR

The reflecting mirror type of illuminator shown in RP266, page 124, figure 3, has been discarded, because of complaints that it provided insufficient illumination at the lower portion of the burette. The feature of the reading shield (fig. 2 of the same reference) has been retained. In one instance this shield has been mounted as was the illuminator of figure 3, but a 3-volt lamp with a ground-glass diffusing screen was placed back of it. In another instance the apparatus was placed within 4 feet of a white wall, and strong light reflected from this was sufficient when the reading shield was used. The new fluorescent daylight lamps of the 30-watt length give good illumination for the necessary observations. Equipped with a strip of black curtain traveling as a belt over pulleys at top and bottom of the lamp, and with a suitable slit in the curtain, which is adjusted to the position of the mercury meniscus, an excellent illuminator can be made at modest cost.

III. MANOMETER-COMPENSATOR

The manometer-compensator has been simplified so that it can be made entirely by the glassblower. Instead of terminating the two electric leads from the manometer with binding posts, fastened in place by cement, these leads, one from the cap and one from the dome of the compensator (see RP266, p. 125, fig. 4), end in small glass cups into which a drop of mercury is put. The wires from the current source are then dipped into this mercury to provide the connection. For some work, the compensator has been modified by projecting the lower tube slightly into the top bulbed portion by means of a ring seal. This construction affords a small ring into which water is put. This serves to supply water from both the top and bottom regions of the compensator tube and so insures proper saturation.[1] The modified manometer-compensator is shown in figure 1.

A miniature neon lamp on a 110-volt circuit may be used to replace the 3-volt miniature lamp across the manometer contact. This will eliminate a battery and condenser.

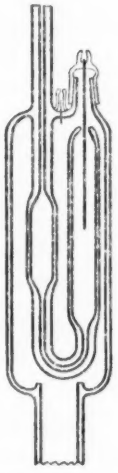

FIGURE 1.—*Modified manometer-compensator (top section only).*

IV. DISTRIBUTOR AND ABSORPTION PIPETTES

In general, no permanent change has been made in the distributor other than the addition of tee cocks to accommodate additional pipettes in experimental models. Experimental study, however, has clearly indicated the desirability of duplicate pipettes for potassium hydroxide and pyrogallate solutions in the case of fuel-gas analysis—one pair for the absorption analysis preceding the combustion, the other for the absorption of carbon dioxide and excess oxygen after the combustion. This arrangement largely avoids significant errors arising from the physical solution of various components in these solutions, and is desirable for exact work, but it increases the number of tee cocks of the distributor from four to six. The 120° cock (RP266, p. 131, fig. 5, cock 9) can be omitted. If a fractional combustion tube is desired, it can be included by adding a single cock of the type illustrated in figure 2.

Further experimental work with solid reagents and small amounts of liquid reagents has disclosed some interesting possibilities, and eventually apparatus to accommodate such reagents will be more fully described. The apparatus so far used is sketched in part in figure 2. Since the small tubes used to hold the reagents act virtually as parts of the distributor, they can properly be discussed at this time. At the beginning of the analysis, the tubes (as well as the distributor) are filled with nitrogen, at the pressure of the compensator. The gas is passed through the tubes by using the combustion pipette as one reservoir and the burette as the other. The stopcocks are bored so that gas may be bypassed, or made to loop the loop, through an absorbing tube. The handle of the cock indicates the bypassed

[1] J. R. Branham, *Saturation by water in gas analysis compensators*, J. Research NBS **18**, 59 (1937) RP962.

position; the position of connection to the tubes is indicated by lines on the key and barrel of the cock. Tubes 1 and 2 are designed for use with small amounts of liquid reagents. These reagents are injected and removed by means of medicine droppers whose nozzles are inserted through the atmospheric bores in the ground caps at top and bottom. The caps are bored diagonally downward to facilitate this operation. The reagent is supported on glass wool, or various forms of glass wicking and cloth, which may be wound upon glass rods to present a long helical path for the gas. Tubes 3 and 4 are designed for solid reagents, and are capped with flat ground plates at bottom and top. These plates may be lubricated and held in place by springs, or cemented in place with a suitable thermoplastic. Tube 4 has been used with a desiccant in performing analyses during which all the gases were

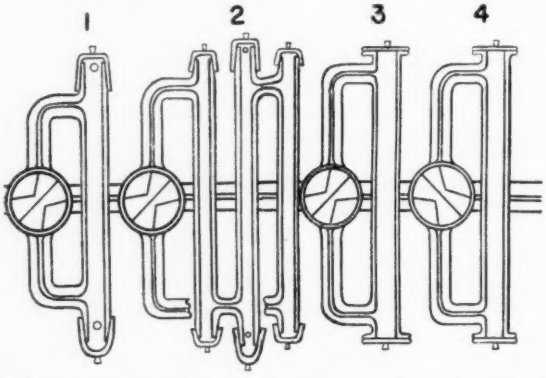

FIGURE 2.—*Apparatus for solid reagents and small amounts of liquid reagents.*

measured dry instead of saturated with water vapor.[2] The same arrangement has been used with a gasometric method for the determination of ethylene oxide in carbon dioxide.[3]

Two new absorption pipettes of the bubbling type have been developed since the description of the one with a perforated platinum plate at the exit (RP266, p. 133, fig. 9). One designed by J. R. Branham and E. O. Sperling, which employs a Pyrex slotted disk, has already been described.[4] The other has been available for some time through the Fish-Schurman Corporation of New York, N. Y., and will probably be obtainable shortly in Pyrex glass. In this model, the gas stream is divided by means of a light-walled sintered glass thimble sealed to the exit (fig. 3). The thimble has been made in two porosities, *G2* and *G3* (see the Fish-Schurman catalog). The thimble of finer porosity was slightly more efficient than the platinum-tipped pipette; that of coarser porosity was slightly less efficient, but proved to be the better choice because it decreases the back pressure. From the practical viewpoint, all three of these distributor pipettes are equally efficient, and all are about equal in cost. The Branham-Sperling type with slotted Pyrex disk is not yet available commercially. From a view-

[2] J. R. Branham, Martin Shepherd, and Shuford Schuhmann, *Critical study of the determination of carbon monoxide by combustion over platinum in the presence of excess oxygen.* (Publication pending.)

[3] J. R. Branham and Martin Shepherd, *Gasometric method and apparatus for the analysis of mixtures of ethylene oxide and carbon dioxide*, J. Research NBS, **22** 171 (1939) RP1175.

[4] J. R. Branham and E. O. Sperling, *Bubbler tip of Pyrex glass for difficult absorptions*, J. Research NBS **22**, 701 (1939) RP1214.

point of general performance, there is little choice. The slotted-disk type is the most rugged of the three; the sintered-thimble type shows the least tendency to clog with repeated absorptions of nearly pure oxygen; and the platinum-tipped type exhibits the least tendency to form slugs of reagent in the inlet tube.

In addition to the above, the pipette shown in figure 4 was designed for use with small samples of gas, say 10 ml, such as are employed in the ordinary Haldane type of analysis. This pipette has a perforated platinum plate 10 mm in diameter.

V. COMBUSTION PIPETTE

The Weaver-Ledig combustion pipette (RP266, p. 136, fig. 10) has been slightly modified. The stopcock at the top (cock 11 of the above-

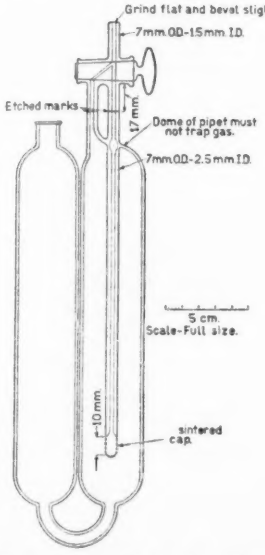

FIGURE 3.—*Absorption pipette with distributor tip of sintered glass.*

FIGURE 4.—*Absorption pipette for small volumes of gas.*

A, Perforated platinum plate.

mentioned figure) has been eliminated, because of the tendency for lubricant from this source to work down into the pipette. The two "stainless" steel caps (2–2 of the same figure) have been provided with 1-mm rods of platinum threaded into the bottom of the cap and extending inside the tapered sleeve of the cap along the longitudinal axis. These rods insure good electric contact if the "stainless" steel becomes corroded. (Products of corrosion can, of course, be removed from inside the original type of cap.)

VI. APPARATUS SUPPORT

Two modifications of the original support (RP266, p. 140) have been made. The first is necessary, the second entirely optional.

The rheostat has been covered so that mercury accidentally spilled will not collect upon wires which are sometimes warm. This is desir-

able in the interest of health, since the danger of too much mercury vapor in air is now well known. An autotransformer can be used instead of the ordinary rheostat.

If it is desired, a sliding support can be substituted for the fixed rings used to support the leveling bulb connected to the burette. A clever arrangement was called to our attention by N. R. White, of this Bureau, and improved by H. W. Bailey, of the Bureau's instrument

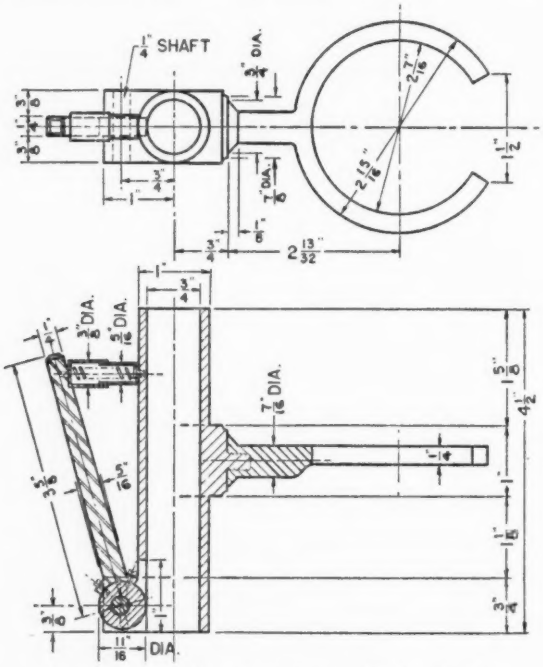

FIGURE 5.—*Sliding support for leveling bulb.*

shop. The sliding ring supporting the leveling bulb is attached to an eccentric cam which bears upon the guide rod and so locks the ring securely in place. The cam is released by pressing a hand lever. The position of the cam is adjustable. The arrangement is shown in figure 5.

These modifications account for new features developed and tried since the first description of our volumetric gas analysis apparatus. They are of sufficient value to pass along to the present or prospective users of this apparatus, but do not constitute any formal change in the original model, other than noted as necessary in the foregoing text. Before announcing definite alterations, further experimental work will be necessary. In general, however, a six-pipette model has proved to be more useful than the four-pipette model, since it permits more accurate work in combustion and additional outlets for special problems.

WASHINGTON, November 14, 1940.